DECOMPOSITION AND ADSORPTION OF PEPTIDES IN ALASKAN COASTAL MARINE SEDIMENTS

by

Honghong Luo

RECOMMENDED:

Augustus J Paul

W. S. Reeburgh

Donald M. Schell

John Braddock

Susan del. Hennichs
Advisory Committee Chair

Susan del. Hennichs
Head, Graduate Program in Marine Sciences and Limnology

APPROVED:

Dean, School of Fisheries and Ocean Sciences

Dean of the Graduate School

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Date

DECOMPOSITION AND ADSORPTION OF PEPTIDES IN ALASKAN COASTAL MARINE SEDIMENTS

A

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Honghong Luo, B. S., M. S.

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Abstract

In organic-rich coastal sediments, hydrolyzable amino acids make up a substantial fraction of the sedimentary content of organic nitrogen. How this organic nitrogen resists decomposition and is preserved in sediments is poorly understood. In order to investigate the factors controlling mineralization and preservation of hydrolyzable amino acids, decomposition and adsorption of peptides were studied in suboxic and anoxic pore water and sediments from Resurrection Bay (RB) and Skan Bay (SB), Alaska. Five tritium-labeled peptides, basic di-lysine, acidic di-glutamic acid, and neutral di-alanine, tri-alanine and hexa-alanine, were used as tracers.

In filtered pore water, the hydrolysis rates were usually low. The exception was that the initial enzymatic hydrolysis of di-alanine and di-glutamic acid was rapid in SB pore water. The hydrolysis rates of both peptides increased with concentration. In sediments, hydrolysis was found to be the rate-limiting step of peptide decomposition. Alanyl and glutamyl peptides were hydrolyzed faster than lysyl peptide, and the hydrolysis rates among alanyl peptides decreased with increasing molecular weight. Peptide hydrolysis was affected more by molecular structure than by oxic or anoxic conditions.

Adsorption of lysyl peptide to sediments was greater than that of other peptides. Basicity enhanced peptide adsorption more than increased molecular weight. Sedimentary organic matter was mainly responsible for peptide adsorption. The different patterns of peptide adsorption in RB and SB sediments were related to the greater total organic carbon concentration in SB sediment. Some of the peptide adsorption was irreversible. Adsorbed peptides were more resistant to biological decomposition than dissolved peptides. Adsorption may be an important step in the process of peptide preservation in sediments, and thus the preservation of sediment organic matter during early diagenesis.

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Chapter 1: Literature Review and Statement of the Problem

Introduction

Accumulation of organic matter in marine sediments is one of the important processes removing carbon from the active earth reservoirs: the atmosphere, ocean, forests, and soils (Schlesinger 1991). Changes in sedimentary organic carbon accumulation over geologic time are thought to be linked to long-term changes in atmospheric carbon dioxide and oxygen content (Berner 1989). On the present-day earth, the rate of sedimentary organic carbon accumulation is highly variable, from less than 0.1 to greater than 1000 g C m^{-2} yr^{-1} (Henrichs 1993). The characteristics of the environment, the sediment, and the deposited organic matter are all important in determining whether an organic substance is remineralized, altered, or preserved unchanged as it is buried (Henrichs 1993).

One model for organic matter decomposition in marine sediments involves the release of soluble organic molecules, such as free amino acids and sugars, by the action of bacterial enzymes. The dissolved compounds are rapidly assimilated and metabolized by the bacteria. Another possible fate of the hydrolysis products is that many organic compounds, including both polar and nonpolar substances, are adsorbed by marine sediments (e.g., Hedges 1977; Shaw *et al.* 1984; Gordon and Millero 1985; Harvey *et al.* 1986; Henrichs and Farrington 1987). In some cases, adsorption slows the decomposition of molecules (Gordon and Millero 1985; Sugai and Henrichs 1992). Also, it has been suggested that dissolved amino acids in sediments undergo melanoidin-type condensation reactions, which lead to refractory geopolymer formation (Nissenbaum 1974; Krom and Sholkovitz 1977; Hedges 1978).

In this study, decomposition and adsorption of peptides in marine sediments were studied in order to explore the processes controlling organic matter accumulation. Peptides with acidic, basic and neutral functional groups and different chain lengths (di-, tri- and hexa-peptides) were used to investigate how the composition of amino acid-containing polymers affects their decomposition and preservation. Skan Bay and Resurrection Bay, Alaska, were chosen as study sites because their sediments have been the subjects of many biogeochemical studies of amino acids and other organic compounds (Reeburgh 1980; Shaw *et al.* 1984; Alperin and Reeburgh 1984, 1985; Alperin 1988; Sugai and Henrichs 1992; Henrichs and Sugai 1993) and because they represent contrasting sedimentary environments. Skan Bay sediment is anoxic and organic-rich, while Resurrection Bay sediment is oxic or suboxic and contains little organic matter.

Decomposition and Preservation of Organic Matter

In marine sediments, the decomposition and preservation of organic materials (OM) are controlled by sediment sources and sedimentary environments. The rates of OM decomposition in marine sediments correlate closely with carbon flux to the sediment surface (Henrichs 1993) and the sediment accumulation rate (Henrichs and Reeburgh 1987). In rapidly accumulating sediments, decomposition rates of OM are high but the efficiency of decomposition is low, 50 - 90% of deposited OM, compared to that in slowly deposited, deep sea sediments, where > 99% of deposited OM decomposes. This means that preservation of OM is greatest where rates of decomposition are highest, a puzzling phenomenon.

Nearly all early diagenetic transformations of OM are due to decomposition by a variety of versatile microorganisms, predominantly bacteria. Bacteria are capable of

decomposing almost any organic compound (Fewson 1988). The intensity of microbial changes is related to the numbers and types of bacteria present. In fine-grained surface sediments, there are 10^5 to > 10^9 bacteria per cm^3, but the numbers of bacteria decline drastically (up to more than 1000 times) with depth within the upper 100 cm (Price 1976; Rheinheimer 1985). With such a reduction in numbers at depth, the intensity of microbial changes is bound to be greatest near the sediment surface.

In surface sediments, where oxygen is available, aerobic bacteria play an important role in OM decomposition, while in deeper layers anaerobic bacteria become more important, as oxygen is depleted with depth. OM is decomposed via different pathways depending upon the availability of electron acceptors (e.g., oxygen or sulfate) for organic matter oxidation. Thermodynamic calculations of energy yield from anaerobic decomposition processes like denitrification, metal oxide reduction, sulfate reduction, and methanogenesis, operating on hypothetical organic molecules, show dramatically lower energy yields than those from oxygen reduction (Froelich *et al.* 1979; Reeburgh 1983). Anoxic conditions are also thought to reduce rates of enzymatic hydrolysis (Meyer-Reil 1991). Anoxic sediments generally have high concentrations of organic matter and accumulate large amounts of organic matter because of high sedimentation rates. This led to the idea that oxidation of sedimentary OM by anaerobic processes is slow and incomplete (e.g., Emerson 1985; Emerson and Hedges 1988).

Bioturbation could also affect the OM preservation or degradation in sediments, for example, by transporting oxidants to depths below the sediment-water interface when burrows are irrigated or by promoting increased microbial activity (Aller 1982; Aller and Yingst 1985). Since the populations of macrofauna responsible for bioturbation decline to zero within about 10 - 20 cm of the oxic-anoxic interface, lack of larger organisms could also be a factor in the greater OM accumulation in anoxic

sediments. Also, Lee (1992) proposed that differences in the numbers and diversity of organisms that graze upon bacteria between oxic and anoxic sediments may explain part of the difference in carbon preservation rate.

There are several reviews relevant to the debate about the rate and efficiency of oxic vs. anoxic decomposition and the role of these processes in carbon preservation (e.g., Henrichs and Reeburgh 1987; Emerson and Hedges 1988; Canfield 1989; Calvert and Pedersen 1992; Henrichs 1993). The fundamental disagreement is that some authors see evidence that anoxic sediments accumulate more of their influx of OM than do oxic sediments which are otherwise similar, while other authors find no conclusive evidence of this. The issue has been difficult to resolve because nowhere in the natural world are there two sediments which differ only in the availability of oxygen.

Another approach to this problem is to decompose OM under controlled oxic or anoxic conditions in the laboratory. A review of recent short-term microcosm and laboratory studies shows that anaerobic rates were not generally intrinsically lower than aerobic rates; fresh organic matter degraded at similar rates under both oxic and anoxic conditions (e.g., Henrichs and Reeburgh 1987; Lee 1992). Experimental evidence has shown that the oxidation of organic matter by sulfate reduction can be as rapid and as efficient as that using oxygen (Foree and McCarty 1970; Jewell and McCarty 1971; Otsuki and Hanya 1972a, b; Jørgensen 1982; Westrich and Berner 1984; Kristensen and Blackburn 1987).

Different constituents of organic matter in sediments have very different decomposition rates, but efforts to understand how sediment OM composition relates to OM preservation are still preliminary. An important reason for this is the composition of most sediment OM is unknown (Engel and Macko 1993). Review of the literature on decomposition rates of different substances in coastal sediments shows a few patterns

(Henrichs 1993). Dissolved, free monomers such as amino acids or sugars decompose on time scales of hours to days. Biogenic polymers or freshly-killed organisms are remineralized over weeks to years. Natural sediment OM, and sedimentary hydrolyzable amino acids, fatty acids, and sterols, appear to decompose at similar rates, with lifetimes ranging from months to about 100 years. Decomposition rates of such sediment constituents appear to be related to sediment accumulation rate. The slowest decomposition rates have been found for refractory polymers such as lignin and melanoidins and for hydrocarbons. It has been proposed that preserved OM in ancient sediments consists almost entirely of intrinsically refractory biogenic polymers (Tegelaar *et al.* 1989), but is difficult to reconcile this idea with the observed relationship between OM preservation and sediment accumulation rates in the modern ocean.

Bacterial Decomposition of Polymers in Seawater and Sediments

Most organic material enters marine sediments as polymeric organic compounds, and the enzymatic hydrolysis of higher-molecular-weight material is an acknowledged initial and rate-limiting step in the utilization of organic matter by microorganisms in aquatic environments and in sediments (Billen 1982; Meyer-Reil 1987; Chróst 1990; Meyer-Reil 1991). Thus, in order to understand decomposition and preservation of sediment organic matter, it is important to understand hydrolysis. Prior to incorporation into microbial cells, polymeric materials undergo step-wise degradation by a variety of cell surface-associated enzymes, or enzymes secreted by intact living cells or liberated into the environment through the lysis of microorganisms. The released oligomers or monomers become available substrates for uptake by microbial cells (Kim and ZoBell 1974; Little *et al.* 1979).

The enzymatic activity in marine sediments has usually been measured by introducing radioactive or dye-labeled substrates, such as methylumbelliferone (MUF) substrates, into sediments (e.g., Meyer-Reil *et al.* 1980; Novitsky 1983; King 1986; Meyer-Reil 1986; Meyer-Reil 1987). However, detailed information on the dynamics and controls of enzymatic hydrolysis is limited, due to the difficulties in the measurement of enzymatic activities in sediments (Oshrain and Wiebe 1979; Sayler *et al.* 1979; Wainwright 1981; Meyer-Reil 1983).

These techniques have also been used to study hydrolytic enzyme activity in seawater, and findings of those studies have some bearing on interpretation of the sediment data. Hollibaugh and Azam (1983) studied the microbial degradation of dissolved radiolabeled proteins in seawater. The results of field observations and laboratory experiments indicated that proteins are degraded by a particle-bound, thermolabile system, presumably bacteria-associated enzymes. Dissolved enzymes are not important in protein degradation in natural seawater samples. Griffith and Fletcher (1991) found that protein availability to nonattached bacteria was low, but adsorbed protein was rapidly hydrolyzed by attached bacteria. In Southern California Bight waters, the turnover rates of protein were found to be of the same order of magnitude as turnover rates of dissolved free amino acids (Hollibaugh and Azam 1983). Heterotrophic bacteria rapidly hydrolyzed peptide analogs in natural water (Somville and Billen 1983). Studies of the chemical form and bacterial utilization of dissolved combined amino acids (DCAA) in estuarine and coastal waters showed that heterotrophic bacteria used DCAA for growth (Hollibaugh and Azam 1983; Hagstrom *et al.* 1984; Tupas and Koike 1990). Although it is not clear how important the DCAA or proteins are in supporting bacterial growth, the coupling of microbial uptake and hydrolysis should control the concentrations of hydrolytic products. Generally, the microflora can immediately

remove the end products of hydrolysis (Güde 1978). The products of extracellular hydrolysis of dissolved proteins do not accumulate in the medium (Hollibaugh and Azam 1983).

The hydrolysis of proteins is dependent on their composition and the concentration of organic substrates. Keil and Kirchman (1993) suggested that the rate of DCAA utilization was different for different pools of DCAA. "Fresh" protein was assimilated rapidly. "Modified" protein, kinetically similar to glucosylated protein, was utilized more slowly than fresh protein, as were combined amino acids that were not in protein. The latter might be present as amino acids adsorbed to clays or associated with humic materials. In these studies, the presence of DFAA inhibited the hydrolysis of DCAA, and the presence of fresh protein inhibited the hydrolysis of modified protein.

Very little is known about the location of enzymatic activity in natural sediments. Extracellular enzymes are enzymes which occur in free form, dissolved in water or adsorbed to surfaces other than those of the producer organism, such as detritus particles or clay surfaces (Burns 1978; Priest 1984; Chróst 1990). Extracellular enzymes act outside the cell. The activity of free extracellular enzymes in interstitial water is usually very low. This may be due to the fact that enzymes excreted by microbial cells into the surrounding water are not of great use to the parent cells; these enzymes may diffuse away or undergo rapid denaturation or decomposition. Most of the extracellular enzymes in sediments are bound to particles or cell surfaces. By adsorption to inorganic and organic particles, enzymes can be physically and chemically immobilized (Burns 1978; Ladd 1978). However, the activity of enzymes adsorbed onto particles is generally decreased or eliminated (Haska 1981; Lorenz and Wackernagel 1987). Microbial cell-bound enzymes may be mainly responsible for the decomposition of the organic material entering sediments, because they gain contact with the substrate following microbial

colonization. However, little is known about the relative contributions of free-living bacteria in interstitial water and bacteria attached to particles to the decomposition of particles in sediments.

In sediments, interfaces are characterized by high enzymatic activity. These interfaces (e.g., sediment/water boundary, walls of macrofaunal tubes and burrows, oxic-anoxic boundary) are known to be zones of high microbial abundance and metabolism (Craven *et al.* 1986). In a study of hydrolysis of glycoside derivatives of MUF in sediments from an intertidal mud flat, King (1986) found that maximal enzymatic activity occurred in surface sediments, with a rapid decline below 2 cm. Also, enzymatic activity underwent strong seasonal variations related to the enrichment of organic material at the sediment surface following sedimentation events.

The products of enzymatic hydrolysis under oxic and anoxic conditions enter different pathways for oxidation and mineralization. However, the influence of anoxic conditions on the activity of hydrolytic enzymes is uncertain (Meyer-Reil 1983; King 1986).

Adsorption and Its Effects on Decomposition of Organic Substances

Mayer (1993) has presented evidence that preservation of OM in marine sediments is due to the physical protection of organic substances within small pores on mineral surfaces. This hypothesis would neatly explain the correlation between sediment OM preservation and sediment accumulation rate. However, there is considerable evidence that the role of adsorption is more complex than Mayer's proposal.

Adsorption may affect both enzyme activity (Lorenz and Wackernagle 1987) and the accessibility of enzyme substrates (Dashman 1977). Adsorption of organic matter onto particle surfaces is a ubiquitous process. There is evidence of adsorption of many

classes of organic compounds to sediments and clay minerals. For example, adsorption of hydrophobic molecules by sediments and clay (Meyers and Quinn 1973; Hedges 1977; Brownawell and Farrington 1986); adsorption of humic matter and related substances (Davis 1982); adsorption of acetate by sediments (Shaw *et al.* 1984; Sansone *et al.* 1987); adsorption of nonprotein amino acids by marine sediments (Henrichs and Farrington 1987); and adsorption of methylated amines (monomethyl-, dimethyl-, and trimethyl amine-) by marine sediments and clay minerals (Wang and Lee 1990) .

There are several studies of the adsorption of amino acids to clay minerals and to marine sediments. The previous results show that adsorption is affected by inorganic and organic constituents of sediments, as well as the structure of amino acids. For both organic-free kaolinite and montmorillonite, the adsorption of basic amino acids was greater than that of acidic and neutral amino acids (Hedges and Hare 1987). Acidic amino acids were only adsorbed by kaolinite, while neutral amino acids were adsorbed to a greater extent by montmorillonite than by kaolinite. In marine sediments, adsorption of basic amino acids (e.g., lysine) was also found to be greater than that of acidic (e.g., glutamic acid) and neutral (e.g., alanine) amino acids (Henrichs and Farrington 1987; Doyle 1988; Henrichs and Sugai 1993). Henrichs and Sugai (1993) found that sediments had a very large adsorption capacity for amino acids compared to the normal dissolved concentrations in pore water.

Dashman (1977) studied the adsorption of peptides by montmorillonite and kaolinite. He found that peptide adsorption was greater on montmorillonite. In general, as the molecular weight, chain length and basicity of peptides increased, adsorption was enhanced; for example, the larger and more basic alanyllysine was adsorbed preferentially to aspartylglycine. Dashman (1977) proposed that adsorption was due to

cation exchange, hydrogen bonding, cation bridge formation, chelation and formation of covalent bonds. However, his data shed little light on the actual adsorption mechanism.

The adsorption of protein on solid surfaces differs from that of DFAA and small peptides due to the special characteristics of protein. Adsorption was found to be greater on hydrophobic as opposed to hydrophilic surfaces. Despite the hydrophobic nature of protein, there are always some polar-amino-acid side chains that can interact strongly with a surface, even if both the protein and surface are negatively charged. Such binding is expected to result in unfolding of the protein and irreversible adsorption. The consequence of this would be the probable exposure of hydrophobic loops to the aqueous solution; adsorption of a second protein layer can therefore reduce the interfacial free energy (McGuire and Krisdhasima 1991). A large amount of experimental evidence supports the concept that once adsorbed, proteins exist in multiple states on a surface (Andrade *et al.* 1984; Horbett and Brash 1987; Jönsson *et al.* 1987; Elwing *et al.* 1988). Kirchman *et al.* (1989) studied the adsorption of two proteins on to Parafilm (a surface with a low work of adhesion and high hydrophobicity) and glass (high work of adhesion and low hydrophobicity). A relationship between work of adhesion and adsorption indicated that hydrophobic interactions between proteins and surfaces were important in determining protein adsorption, especially at high salinity. For all surfaces, adsorption was higher in seawater than in a low ionic strength buffer. Protein adsorption by particles decreases with increasing surface energy (Samuelsson and Kirchman 1990).

The adsorption of organic compounds in sediments has been shown to decrease the availability of organic material to microorganisms in a few studies. By this effect, adsorption could influence the distribution of organic compounds in sediments (Carter 1978; Christensen and Blackburn 1980) and affect organic matter cycling in marine

sediments (Burdige and Martens 1990). For example, soil scientists have studied the adsorption of amino acids, peptides, and proteins by clay minerals and found that adsorption decreases metabolism by soil microorganisms (e.g., Marshman and Marshall 1981). Several studies have reported that the adsorption of organic compounds serves to protect them from biodegradation by marine bacteria (Christensen and Blackburn 1982; Gordon and Millero 1985; Sugai and Henrichs 1992). Gordon and Millero (1985) investigated the effect of adsorption by hydroxyapatite on the biodegradation rate by a sediment bacterium, *Vibrio alginolyticus*. They found that adsorption was least for glucose and acetic acid, intermediate for glutamic acid, and greatest for citric acid; the respiration and assimilation of these substrates was inversely correlated with adsorption. Harvey *et al.* (1986) found that membrane lipid biodegradation in marine sediments was inversely correlated with adsorption to sediment organic matter. Marshman and Marshall (1981) and Henrichs and Doyle (1986) found that amino acids incorporated into melanoidins resisted microbial decomposition in soils and sediments. Dashman and Stotzky (1986) found that the inhibition of protein degradation by adsorption probably caused the lower bacterial growth on protein when clay was present.

Free amino acids are rapidly adsorbed by marine sediments, and the strongly adsorbed amino acids decompose much more slowly than those which are adsorbed less (Doyle 1988; Sugai and Henrichs 1992). Adsorption of amino acids is rapid and results in a marked decrease in the decomposition rate, at least on time scales of hours to weeks (Christensen and Blackburn 1980; Doyle 1988).

Some studies have concluded that sedimentary organic matter played an important role in the adsorption of amino acids and acetate (Rosenfeld 1979; Sansone *et al.* 1987). Organic substances in sediment from Resurrection Bay, Alaska (Henrichs and Sugai 1993) were also suggested as the primary adsorber of free amino acids because

very little clay was present in the sediment. Wang and Lee (1993) found that adsorption of amino acids by sediments decreased substantially after peroxide pretreatment or pre-extraction with seawater or sodium hydroxide.

Ion-exchange was found to be an important process in amino acid adsorption. Also, a decrease in adsorption after sodium borohydride or sodium bisulfite pretreatment of the sediment supported the notion that a melanoidin-type reaction might be responsible, in part, for adsorption (Henrichs and Sugai 1993). Melanoidin-type condensation reactions, leading to the formation of a refractory geopolymer, have been proposed as a pathway of formation of sedimentary humic and fulvic acids (Nissenbaum 1974; Krom and Sholkovitz 1977). Abelson and Hare (1971) found that the adsorption of amino acids (with different functional groups) by natural and artificial humic acids and kerogens was similar to that by sediments and clays, i.e., basic > neutral > acidic. Hedges (1978) reported that the basic amino acid lysine reacted with glucose much more rapidly than glutamic acid (acidic) or valine (neutral) to form melanoidin-type polymers. Also, Doyle (1988) found that mixing alanine with a fulvic acid extract of sediment significantly reduced its rate of mineralization.

The decomposition and adsorption processes of free amino acids by sediments have probably received more attention than adsorption of any other specific group of molecules. However, most amino acids in sediments are not free or readily extractable, but are apparently bound in peptide linkages. These are hydrolyzed by 6 N HCl after 24 h at 100°C. Hydrolyzable amino acids make up a substantial fraction of total sediment organic carbon and, especially, organic nitrogen. For example, in coastal Peru sediments (Henrichs *et al.* 1984), they constitute up to 30% of organic carbon and 70% of organic nitrogen. More typically, in estuarine and shelf sediments, they make up 10% of the organic carbon and 25% of the organic nitrogen. Thus, study of decomposition and

adsorption of amino acid-containing polymers is necessary for understanding of the preservation of bound amino acids in sediments, and, more generally, the preservation of organic matter in marine sediments.

Statement of the Problem

This study investigated how peptides are decomposed and adsorbed in marine sediments, how molecular structures of peptides and sedimentary environments affect decomposition and adsorption, and how adsorption affects the preservation of peptides.

Hypotheses My first hypothesis is that the decomposition of adsorbed peptides in sediments is slower than the decomposition of dissolved peptides. This hypothesis is based on prior observations of the decomposition of adsorbed free amino acids.

My second hypothesis is that the adsorption mechanism and behavior of peptides in sediments should be between those of free amino acids and proteins.

My third hypothesis is that the organic matter on surfaces of sediment particles is a plausible source of adsorption sites for amino acid polymers in marine sediments. There is strong evidence that organic matter is the main adsorber of amino acids in several coastal sediments.

My fourth hypothesis is that the decomposition rates of the same added peptides in oxic and anoxic sediments (Resurrection Bay and Skan Bay sediments, respectively) are similar. This is probably because factors other than the presence of oxygen control the rates of peptide hydrolysis and mineralization.

Significance In marine sediments, hydrolyzable amino acids are major constituents of the organic matter (Henrichs *et al.* 1984) and are quite stable during early diagenesis. However, the reasons why they escape bacterial decomposition are unknown

(Henrichs and Reeburgh 1987). The results of this study will help to determine how sediment and peptide characteristics affect the preservation of amino acid-containing polymers during early diagenesis. In a broader sense, this work addresses the factors which lead to preservation of sediment organic matter.

Chapter 2: Decomposition and Adsorption of Peptides in Skan Bay Sediment

Abstract

The decomposition and adsorption of five C-terminal ^{3}H-labeled peptides, di-lysine, di-glutamic acid, di-alanine, tri-alanine and hexa-alanine, were studied in pore water and sediments from Skan Bay, Alaska. The decomposition of ^{3}H-peptides involved two steps: the hydrolysis of the peptide to the corresponding free amino acids and the respiration of amino acids to ^3H_2O. The hydrolysis rate was concentration-dependent in both pore water and sediments, and hydrolysis was the rate limiting step in the decomposition of ^{3}H-alanyl and ^{3}H-glutamyl peptides in sediments. ^{3}H-Di-glutamic acid and ^{3}H-di-alanine were rapidly hydrolyzed in both extracted pore water and sediments, while ^{3}H-di-lysine, ^{3}H-tri-alanine and ^{3}H-hexa-alanine were hydrolyzed more slowly in extracted pore water than in sediments. The hydrolysis rate of ^{3}H-di-glutamic acid was greater than that of other ^{3}H-peptides. The rate constants of hydrolysis decreased in the order: di-glutamic acid $\geq$ di-alanine > tri-alanine $\geq$ hexa-alanine > di-lysine. The ^{3}H-alanyl peptides were respired faster than ^{3}H-di-glutamic acid and ^{3}H-di-lysine in sediments due to the rapid respiration of their hydrolytic product, alanine.

To examine peptide adsorption, the hydrolytic enzyme activity in sediments was eliminated by autoclaving. Adsorption partition coefficients of ^{3}H-peptides were constant over a wide concentration range and varied in the order: di-lysine > di-alanine > di-glutamic acid > tri-alanine > hexa-alanine. Saturation of adsorption sites in sediments was not observed within the solubility range of peptides in seawater. Basicity of peptides and characteristics of the sediment surface were more important than molecular weight

of peptides in affecting adsorption. The adsorption was by ion exchange or binding to sediment organic substances, and the adsorbed peptides could be completely recovered by acid hydrolysis. Adsorption of peptides onto sediment particles partially protected them from biodegradation.

Introduction

Microorganisms in benthic ecosystems play a key role in the decomposition of sediment organic material. Most of the organic material enters marine sediments as polymeric organic compounds, but microbial cells can directly utilize only the low-molecular-weight organic material, not polymeric organic compounds (Kim and ZoBell 1974; Little *et al.* 1979). Extracellular enzymes secreted from living cells or liberated through lysis of cells are required to degrade the polymeric material into oligomers or monomers that can be taken up by microorganisms and used as sources of energy or incorporated into biomass.

Many organic compounds are adsorbed to surfaces in sediments. Adsorbed organic matter makes nearly all particle surfaces in nature negatively charged (Neihoff and Loeb 1972; Hunter 1980; Davis and Gloor 1981; Tipping and Cooke 1982). Adsorption can be as rapid as bacterial uptake, as shown by a study of protein degradation (Kirchman *et al.* 1989). Therefore, decomposition and adsorption are two important and competing processes in the early diagenesis of polymeric compounds.

The enzymatic hydrolysis of polymers is generally considered to be the rate-limiting step in organic carbon oxidation in sediments (Meyer-Reil 1991). Enzymatic hydrolysis in marine sediments has been studied using radiolabeled substrates and methylumbelliferone (MUF) substrate analogs (e.g., Meyer-Reil *et al.* 1980; Novitsky 1983; Meyer-Reil 1986; King 1986; Meyer-Reil 1987). However, detailed information

on the dynamics and controls of enzymatic hydrolysis is limited due to the difficulties in the measurement of enzymatic activities in sediments (Oshrain and Wiebe 1979; Sayler *et al.* 1979; Wainwright 1981; Meyer-Reil 1983).

Adsorption of organic compounds in sediments may decrease their availability to microorganisms (Christensen and Blackburn 1980; Marshman and Marshall 1981; Christensen and Blackburn 1982; Gordon and Millero 1985; Dashman and Stotzky 1986; Doyle 1988; Sugai and Henrichs 1992). There have been many studies of the adsorption of amino acids to inorganic and organic constituents of sediments. For example, Henrichs and Sugai (1993) investigated the adsorption of amino acids by sediments from Resurrection Bay, Alaska. They found that dissolved amino acids were rapidly removed from solution. Sediments had a very large adsorption capacity for amino acids compared to the normal dissolved concentrations in pore water. Adsorption was enhanced by increased basicity of amino acids. A substantial fraction of amino acid adsorption was not reversible in concentrated amino acid solutions. Ion exchange and condensation reactions were suggested as processes of adsorption. Adsorption of amino acids was rapid and resulted in a marked decrease in the decomposition rate, at least on time scales of hours to weeks (Sugai and Henrichs 1992).

However, there is a dearth of studies of peptide adsorption and decomposition in sediments, although most amino acids in sediments are not free or readily extractable but are largely bound in peptide linkages (e.g., Henrichs *et al.* 1984). The limited information on the adsorption and decomposition of peptides and protein on clays and marine particles (Dashman 1977; Kirchman *et al.* 1989) indicates that adsorption can be rapid compared with the rate of protein degradation by bacteria, and bacteria grow more slowly on adsorbed peptides and protein than on the dissolved substances. Also, the

extent of adsorption is related to peptide composition, molecular weight, and the surface energy of the particle surface (Samuelsson and Kirchman 1990).

In this study, the decomposition and adsorption processes of peptides in marine sediments and pore water were investigated. The purpose was to examine how peptide composition affects decomposition (including enzymatic hydrolysis and respiration) and adsorption, and how adsorption influences decomposition. This study will potentially help to explain the preservation of amino acids bound in sediments, which remains a poorly-understood phenomenon (Henrichs and Reeburgh 1987).

Materials and Methods

Study site The sediments examined in this study were taken in July 1990 and October 1992 from Skan Bay (53°37'N, 167°03'W), a fjord of 65 m maximum depth and 10 m sill depth on the northwest side of Unalaska Island in the Aleutian chain (Figure 2.1). Skan Bay sediments are permanently anoxic and organic-rich (5-6% TOC), apparently due in part to large inputs of organic matter derived from kelp (Alperin 1988). The water column overlying the sediment is oxygen depleted or becomes anoxic in the late fall. Skan Bay has been the site of previous biogeochemical studies (Reeburgh 1980; Shaw *et al.* 1984; Alperin and Reeburgh 1984, 1985; Alperin *et al.* 1992).

Sampling on board ship Sediment samples were collected with a box corer. After removing the overlying water, plates with holes at about 4.5 - 6 cm depth were inserted vertically into the mud. Subsamples of intact sediment were collected for decomposition experiments by inserting 10 to 12 mL plastic syringes or glass incubation syringes horizontally into the plate holes. After filling, the plastic syringes were sealed with rubber stoppers and glass syringes were sealed with Teflon-faced silicone septa and aluminum crimp seals. Sediment samples for adsorption experiments were collected

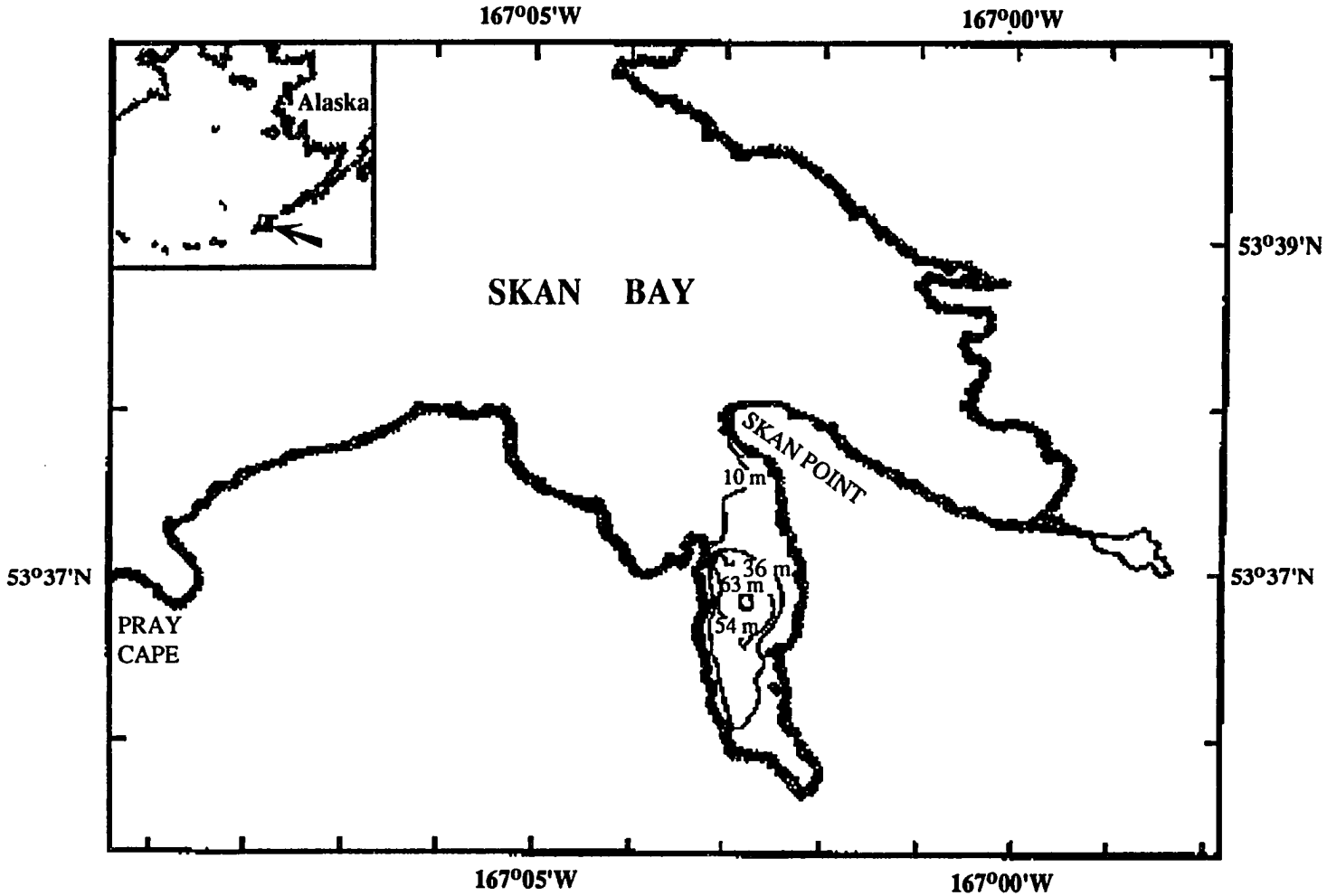


Figure 2.1. Map of Skan Bay.

from box cores by manually scooping the 4.5 - 6 cm layer into mason jars. The mason jars were sealed and stored in an incubator (2 - 4°C) for experiments conducted in our Fairbanks laboratory.

Standard peptide solutions The tritiated peptides, di-glutamic acid (glu_2), di-alanine (ala_2), tri-alanine (ala_3), hexa-alanine (ala_6) and di-lysine (lys_2), used in this study were synthesized by extending the C-terminal end of commercially available non-labeled peptides by azide coupling with tritiated amino acids (i.e., [3, 4-^{3}H] glutamic acid, [2, 3-^{3}H] alanine, [4, 5-^{3}H] lysine) (Bodanszky and Bodanszky 1984a, b). The labeled peptides were separated and purified by thin-layer chromatography and high performance liquid chromatography (HPLC) (Iskandarani and Pietrzyk 1981) using a reversed phase column (Figure 2.2a, for details of the procedure see APPENDIX II). In each synthesized product, 60 to 86% of tritium activity was ^{3}H-peptide and the remainder was the corresponding ^{3}H-amino acid. The specific activities of synthesized ^{3}H-glu_2, ^{3}H-ala_2, ^{3}H-ala_3, ^{3}H-ala_6 and ^{3}H-lys_2 were 152.1, 399.5, 276.2, 133.1 and 246.8 mCi/mg, respectively. The decomposition of the C-terminal residue could be monitored by using these peptides.

Non-labeled peptides (Sigma Chemical Company) were dissolved in filtered (Whatman GF/F filter) seawater to make solutions of the concentrations needed for experiments. ^{3}H-peptides dissolved in glass-distilled water (less than 300 μL) were added into non-labeled peptide solutions (about 50 to 70 mL) at a concentration of 10^4 dpm/mL.

High Performance Liquid Chromatography The separation of ^{3}H-peptides from ^{3}H-free amino acids and ^3H_2O, as well as the measurements of peptide concentrations, were conducted using reversed-phase HPLC. The analytical columns

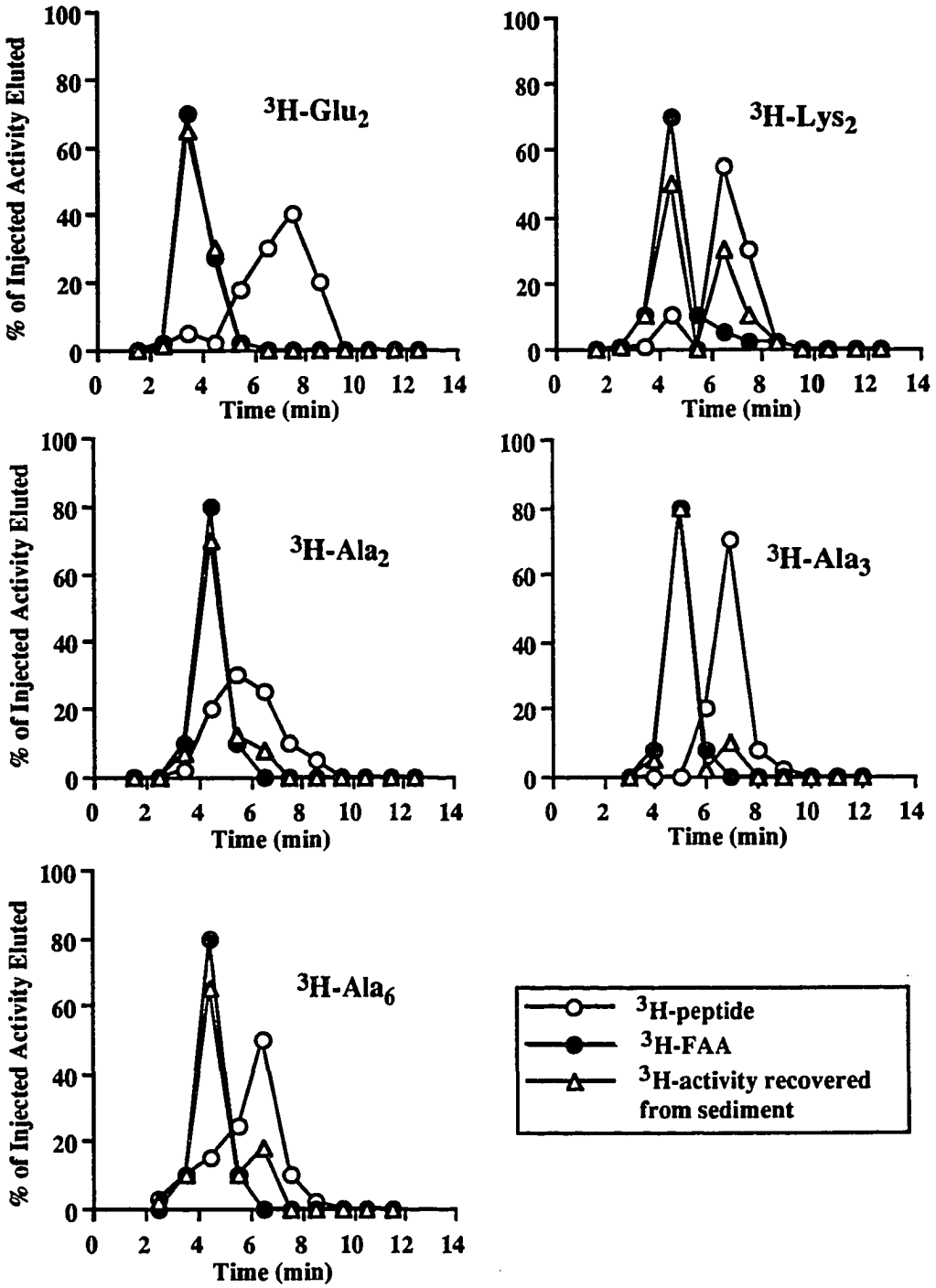


Figure 2.2a. HPLC Separation and Analysis of ^{3}H-Peptides and ^{3}H-Free Amino Acids. Mobile phase: (A) 0.1% acetic acid adjusted with 6N HCl to pH 1.4 (B) methanol. Linear solvent gradient: 100% A for 5 minutes, then from 100% A to 100% B in 40 minutes.

were Lichrosorb RP-18 (250 mm x 4.0 mm, 10 μm particle size) and Econosphere C18 (250 mm x 4.6 mm, 5 μm particle size).

For the separation of ^{3}H-fractions, 1 to 1.5 mL of solution was injected after filtration through a 0.2 μm Nuclepore filter. The acidic extracts were neutralized with 6 N sodium hydroxide (NaOH) solution before filtration. The mobile phases were methanol and 0.01% acetic acid adjusted with 6 N HCl to pH 1.4, or methanol and 0.03% acetic acid (Iskandarani and Pietrzyk 1981; Mant and Hodges 1991). Fractions of the eluate containing ^{3}H-activity were collected. Under the HPLC conditions shown in Figure 2.2a, the ^{3}H-peptides were separated from related ^{3}H-free amino acids (FAA). Since $^{3}H_2O$ was eluted at the same time as the ^{3}H-FAA, it was separated from other ^{3}H-fractions by distillation. Ten mL of liquid scintillation solution (UniverSol, ICN Biomedical, Inc.) was added to each collected fraction. The radioactivity was measured by a liquid scintillation counting using Beckman Model LS 3801, after waiting at least several hours to minimize chemiluminescence.

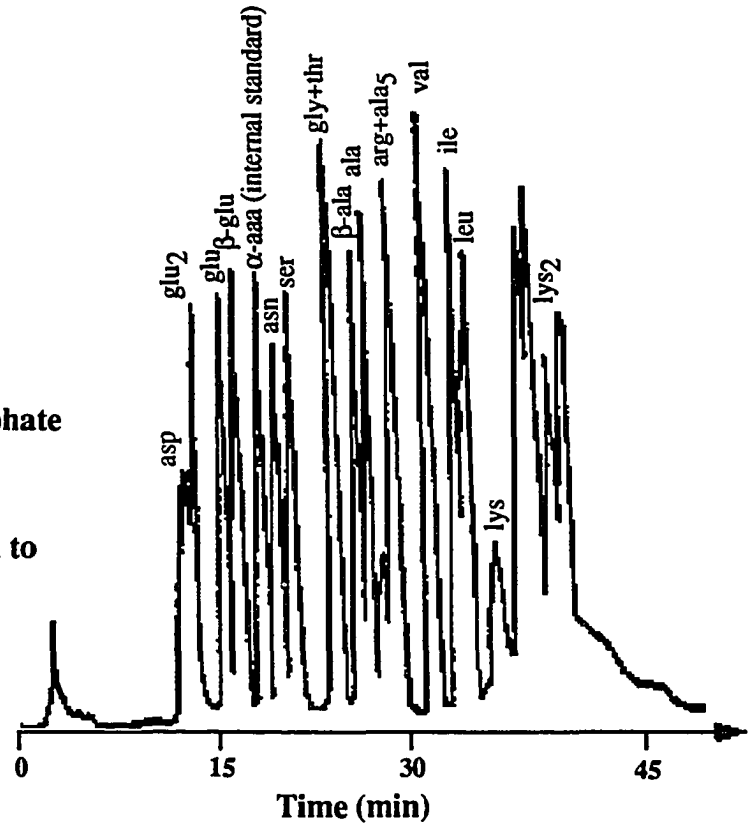
Peptide concentrations were determined by precolumn fluorescence derivatization of peptides with *o*-phthaldialdehyde/α-mercaptoethanol (OPT) reagent (Lindroth and Mopper 1979; Jones *et al.* 1981). Fifty mg of *o*-phthaldialdehyde and 100 mg dodecyl sulfate were dissolved in 5 mL methanol, and then 5 μL α-mercaptoethanol was added to make the OPT reagent. OPT was allowed to "age" for 3 to 4 days. Peptides were reacted with 50 μL OPT reagent and 100 μL 0.4 M sodium borate buffer at room temperature for 2 minutes. Samples were filtered (0.4 μm GF/F) before derivatization. For samples containing high concentrations of ammonia, the filtrate was stripped of ammonia at 80°C for 30 minutes before derivatization. Phosphate buffer (0.02 M, pH 6.8) and methanol or phosphate buffer and acetonitrile were employed as mobile phases. Five peptide standards were separated from the free amino acids which usually were also

present in pore water (Figure 2.2b). The peptides had fluorescence response/mole ratios 3 to 30 times lower than those of the related free amino acids, and the response factors of glu_2, ala_3 and ala_5 were about 3 times lower than those of lys_2 and ala_2 (APPENDIX III, Table A.1).

Decomposition of peptides in pore water Sediment was transferred from a plastic syringe into a centrifuge tube on board ship. For experiments in Fairbanks, sediment was transferred from mason jars under nitrogen. After centrifugation at -1°C and 2500 rpm for 10 minutes, the pore water was filtered through a 0.4 μm GF/F filter or 0.2 μm Nuclepore filter. The filtered pore water was degassed under argon or nitrogen for 20 minutes. ^{3}H-peptide solutions were added and mixed with pore water in a volume ratio of 1:2. During the long-term incubation (up to 116 hours), the mixture was frozen at each time point, and it took about 15 minutes for 10 mL of pore water to freeze. For the short-term incubations (< 1 hour), the mixture was placed in boiling water for 60 sec to stop further decomposition, then cooled in an ice bath for 60 sec before freezing or HPLC analysis.

Decomposition experiments on intact sediment The decomposition studies were carried out on board ship, except for the HPLC analyses (APPENDIX IV, Figure A.1a). Ten to 12 mL of sediment were transferred to a 50 mL centrifuge tube under argon gas. Three mL of the 0.1 μM ^{3}H-peptide solution was added and mixed for 1 minute, then incubated at 2 to 4°C for 0, 1, 5, 24, and 48 hours. After incubation, the slurry was centrifuged at -1°C for 10 minutes and the supernatant vacuum filtered through a 0.4 μm GF/F filter. One mL of the filtered supernatant was mixed with 10 mL scintillation fluid and allowed to sit at least overnight before liquid scintillation counting. The rest of the filtrate was frozen at -30°C. The ^{3}H-peptide and amino acid in the filtrate were separated by HPLC and counted within a month after the cruise.

Mobile phase: (A) phosphate buffer, 0.02 M, pH6.8; (B) methanol.
Solvent gradient: linear gradient from 100% A to 100% B in 40 minutes.



Mobile phase: (A) phosphate buffer, 0.02 M, pH6.8; (B) acetonitile/phosphate buffer.
Solvent gradient: linear gradient from 100% A to 100% B in 40 minutes.

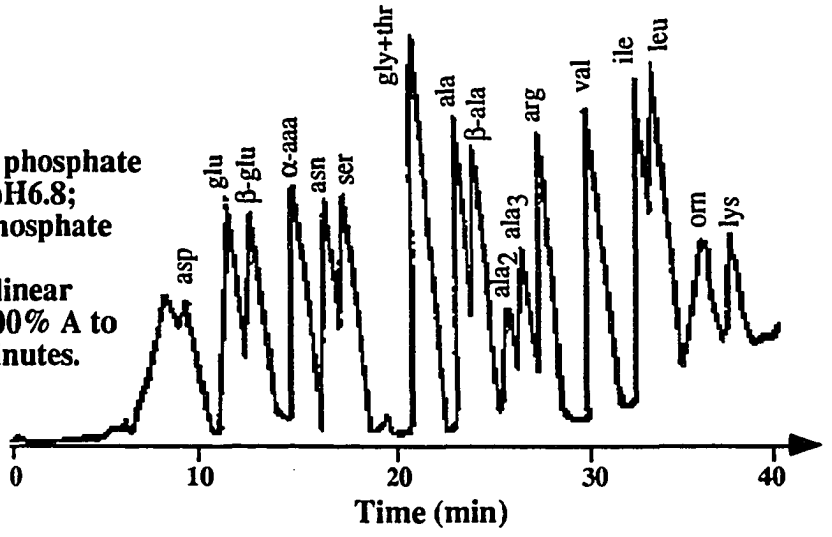


Figure 2.2b. Gradient Separation of Peptides and Amino Acids.

Killed controls for sediments Several treatments were used in an effort to stop biological activity in sediments. For the formalin treatment (done only during July 1990), 30 μL of formalin was injected into each 10 mL syringe containing sediment, and stored at 3°C for at least 12 hours before use. In the chilling treatment, sediment in plastic syringes was chilled at -1.5°C for 2 hours. For heat treatment, sediment sealed in glass syringes or mason jars was heated at 69°C or 85°C for 3 hours. For autoclaving, sediment sealed in a mason jar (250 or 500 mL) was cooked in a pressure cooker at 15 p.s.i. for 1 or 2 hours, and rinsed 3 to 5 times with half of the sediment volume of filtered (0.2 μm Nuclepore filter) seawater. After the final rinse, seawater was added to the sediment to compensate for the pore water loss during the centrifugation.

Adsorption experiments The adsorption experiments were conducted in autoclaved sediments using both nonlabeled and labeled peptides (APPENDIX IV, Figure A.1a). The solution concentrations were 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300, and 1000 μM. The adsorption experiments were also conducted in untreated sediments using nonlabeled peptides with concentrations of 0.03 μM.

^3H_2O was added to the nonlabeled peptide solutions to evaluate the mixing efficiency of the added solution with pore water. The added solutions were effectively homogenized with pore water with 96.2±2.5% (n=72) of added tritium activity being recovered.

Exchange and extraction experiments All solutions in these experiments were prepared with filtered seawater (0.2 μm Nuclepore filters). Seawater, 1 mM sodium acetate and 1 mM cesium chloride (CsCl) solutions were used to extract the adsorbed peptides, presumably via an ion exchange process. These solutions will be referred to as exchange solutions. Acid (1 N HCl) and base (0.6 N NaOH) were also used, and will be referred to as extraction solutions (for detail see APPENDIX IV,

Figure A.1b). After conducting adsorption experiments on sediment samples, three to five mL of the exchange or extraction solutions were added to the sediment pellet for 2 minutes (the solution concentrations were diluted to half of the original ones), then centrifuged. The supernatant was vacuum filtered. One mL of the filtered supernatant was mixed with 10 mL scintillation fluid and allowed to sit at least overnight before liquid scintillation counting. The rest of the filtrate was frozen at -30°C. The ^{3}H-fractions in the filtrate were separated by HPLC and counted within a month after the cruise.

Decomposition of adsorbed peptides To examine how adsorbed ^{3}H-peptides were biologically decomposed (APPENDIX IV, Figure A.1c)., three mL of 0.1 μM ^{3}H-peptide standard solutions were first incubated in 10 to 12 mL of autoclaved sediment for 1 hour to ensure that ^{3}H-peptide was adsorbed but decomposed. After centrifugation and filtration, the sediment, with adsorbed peptides, was mixed with 3 mL seawater and 10 to 12 mL of fresh sediment for 1 minute under argon gas, and then incubated at 2 to 4°C for 0, 1, 5, 24, and 48 hours.

Calculation of parameters The decomposition of ^{3}H-peptides was evaluated as the percent of added peptide activity recovered as ^{3}H-FAA and ^3H_2O. The rates of ^{3}H-peptide hydrolysis and respiration were calculated in units of nanomoles of ^{3}H-peptide per cm^3 of pore water (pw) or sediment per day.

In sediments,

$$\%\ \text{of}\ ^3\text{H-peptide respiration} = 100\times \frac{[^3H_2O\ \text{in pw}] - [^3H_2O\ \text{in added solution}] - [^3H_2O\ \text{respired from added}\ ^3\text{H-FAA}]}{[^3\text{H-peptide in added solution}]} \quad \text{----[1]}$$

where [^3H_2O respired from added ^{3}H-FAA] can be calculated according to the mineralization rates of free amino acids in Skan Bay sediments (Sugai and Henrichs, in preparation).

$$\% \text{ of hydrolysis} = 100x \frac{[^3H_2O \text{ produced}]R_1 + [^3\text{H-FAA in pw}] + [^3\text{H-FAA in AE}]R_2 - [^3\text{H-FAA added}]}{[^3\text{H-peptide added}]} \quad \text{----[2]}$$

where [^3H_2O produced] is the [^3H_2O in pw] corrected for the [^3H_2O in added solution]. As 14 to 40% of the activity in added solution was ^{3}H-FAA, the ^{3}H-FAA in the added solution must be considered in the calculation of the ^{3}H-peptide hydrolysis. Assuming that all the ^3H_2O in pore water is produced by the respiration of ^{3}H-FAA, the calculation of hydrolysis in equation (Eq.) [2] included not only the ^{3}H-FAA remaining in pore water, but also the ^{3}H-FAA adsorbed to sediments and that respired to ^3H_2O. The fraction of hydrolyzed ^{3}H-FAA adsorbed can be estimated by measuring the ^{3}H-FAA in the acid extract. R_1 is an estimate of the following ratio for the amino acid when taken up by bacteria:

$$R_1 = \frac{[^3H_2O \text{ produced}] + [\text{biomass produced}]}{[^3H_2O \text{ produced}]} = 1 + \frac{[\text{biomass produced}]}{[^3H_2O \text{ produced}]}$$

$$= 1 + \frac{[^3\text{H added}] - [^3\text{H in pw}] - [^3\text{H in AE}]}{[^3H_2O \text{ produced}]}$$

R_2 is an estimate of the total adsorption: reversible adsorption ratio of ^{3}H-FAA, when reversibly adsorbed FAA are those extracted by acid solution:

$$R_2 = \frac{[\text{irreversible}] + [\text{reversible}]}{[\text{reversible}]}$$

For the calculation of hydrolysis at the initial time point (15 to 18 minutes) in Eq. [2], if the data on [^{3}H-FAA in AE] are not available, Eq. [2] can be changed to:

$$\% \text{ of hydrolysis} = 100 \times \frac{[^3\text{H-FAA in pw}] - [^3\text{H-FAA added}]\,A}{[^3\text{H-peptide added}]} \quad \text{----[3]}$$

where A is the fraction of the added free amino acid remaining in pore water after 15 minutes. A varied with the added amino acid concentration (Sugai and Henrichs, in preparation). After 15 minutes, there was no respiration of ^{3}H-peptides. The calculation in Eq. [3] does not consider adsorption of the hydrolyzed ^{3}H-FAA. The differences in the calculated hydrolysis between Eqs. [2] and [3], for experiments in which [^{3}H-FAA in AE] was measured, were of the same magnitude as the variation among replicate samples, indicating that the assumption of no FAA adsorption had little impact on the results.

The fraction of ^{3}H-peptide adsorbed and the fraction of the adsorbed ^{3}H-peptide exchanged or extracted were calculated using the following equations:

$$\% \text{ of adsorption} = 100 - \% \text{ of hydrolysis} - \% \text{ of remaining in pore water} \quad \text{----[4]}$$

$$\% \text{ of adsorbed exchanged} = 100 \times \frac{\% \text{ of exchange}}{\% \text{ of adsorption}} \quad \text{----[5]}$$

$$\% \text{ of adsorbed extracted} = 100 \times \frac{\% \text{ of extraction}}{\% \text{ of adsorption}} \quad \text{----[6]}$$

In the experiments with extracted pore water, the fraction of peptide respiration can be calculated using Eq. [1]. The term [^3H_2O respired from added ^{3}H-FAA] can be omitted because the respiration of ^{3}H-amino acids in pore water was very slow over the incubation time used in this study (McDaniel 1989). The fraction of hydrolysis at each incubation time point was calculated using Eq. [3], with A = 1. For short incubations, the time used in calculating the hydrolysis rate includes the time of mixing.

Clay mineral analysis Wet Skan Bay sediment was rinsed with distilled water and treated with hydrogen peroxide to oxidize organic matter. The less than 2 μM fraction was collected by centrifuging the sediments at 1000 rpm for 4 minutes and removing the supernatant containing suspended clays.

Results

By HPLC analysis, almost all of the activity in pore water or extracts was identified as either the added ^{3}H-peptide or its decomposition products, an ^{3}H-amino acid or ^3H_2O. There was no significant accumulation of other dissolved substances, such as volatile fatty acids. The decomposition of peptides is through free amino acids to CO_2 and H_2O, and it can be evaluated by measuring the changes in ^{3}H-peptide, ^{3}H-free amino acid and ^3H_2O compositions in pore water.

Decomposition of ^{3}H-peptides in pore water The stability of the ^{3}H-peptides in filtered pore water varied with their structure. ^{3}H-ala$_3$ and ^{3}H-ala$_6$ were neither hydrolyzed nor respired, and only 5.9±5.7% of the added ^{3}H-lys$_2$ activity was hydrolyzed after a 24-hour incubation (Figure 2.3). In contrast, the hydrolysis of ^{3}H-glu$_2$ and ^{3}H-ala$_2$ was rapid; 33 to 62% of the added ^{3}H-glu$_2$ over the concentration range of 0.01 μM to 30 mM (Figures 2.3, 2.4 and 2.5) and 49% of ^{3}H-ala$_2$ at 0.03 μM (Figure 2.3) were hydrolyzed after 15 minutes. However, after this rapid initial hydrolysis, the peptide and amino acid concentrations did not change significantly for 24 hours. The hydrolyzed fraction of ^{3}H-glu$_2$ began to increase after 70 hours (Figure 2.5). Only ala$_2$ was respired to a measurable extent within 24 hours, with 12% of the added activity found as ^3H_2O after 5 hours. The observed respiration of ^{3}H-ala$_2$ probably was due to the respiration of ^{3}H-alanine.

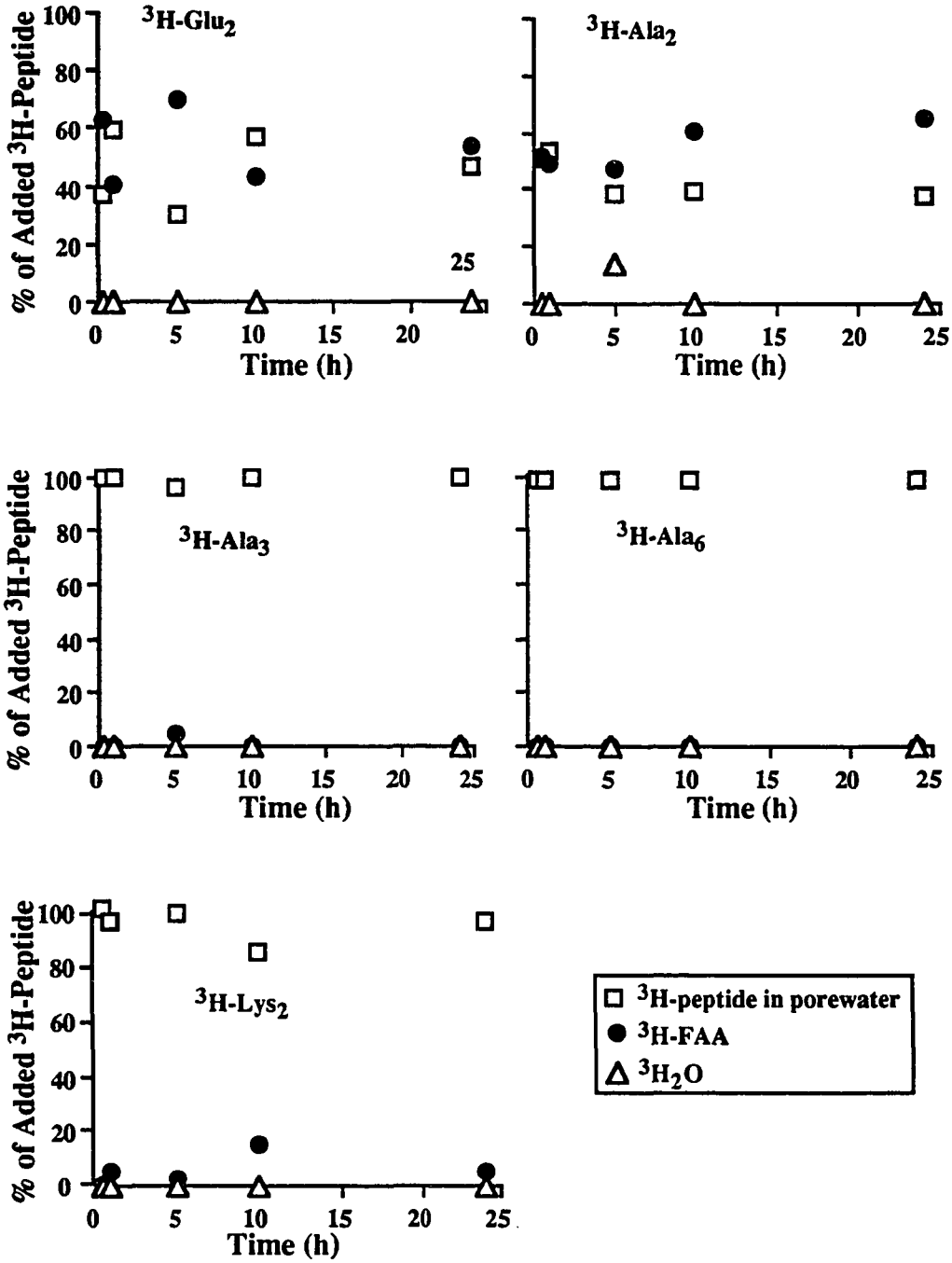


Figure 2.3. Decomposition of ^{3}H-Peptides in Skan Bay Pore Water.

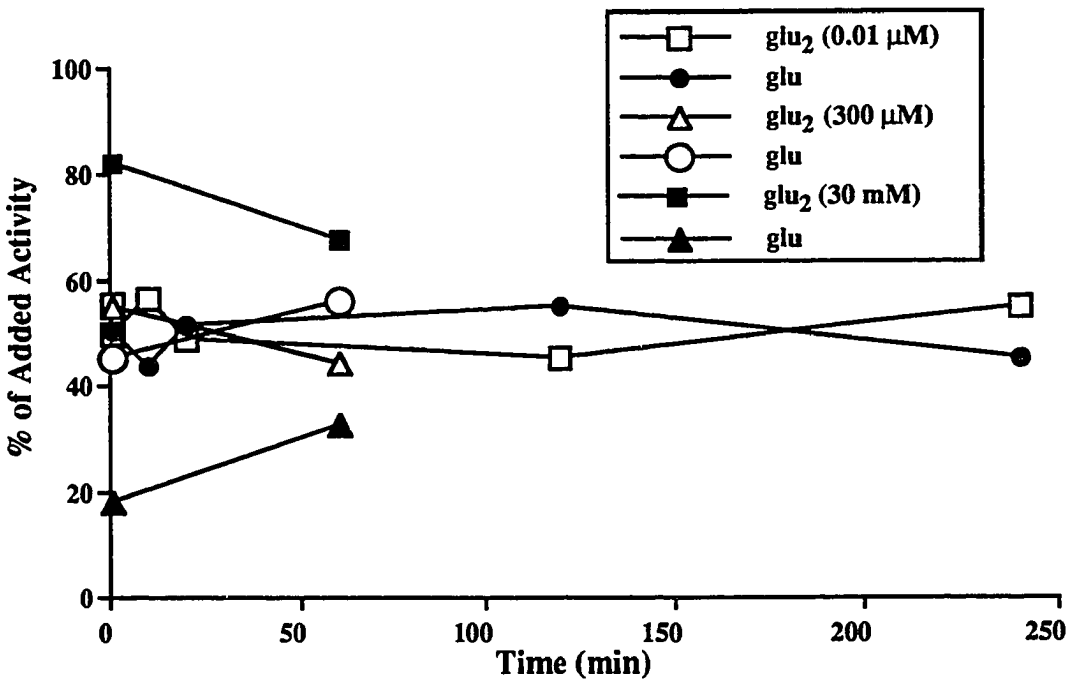


Figure 2.4. Decomposition of ^{3}H-Glu$_2$ in Skan Bay Pore Water. Concentrations indicated in parentheses are the initial dissolved peptide concentration in pore water before decomposition.

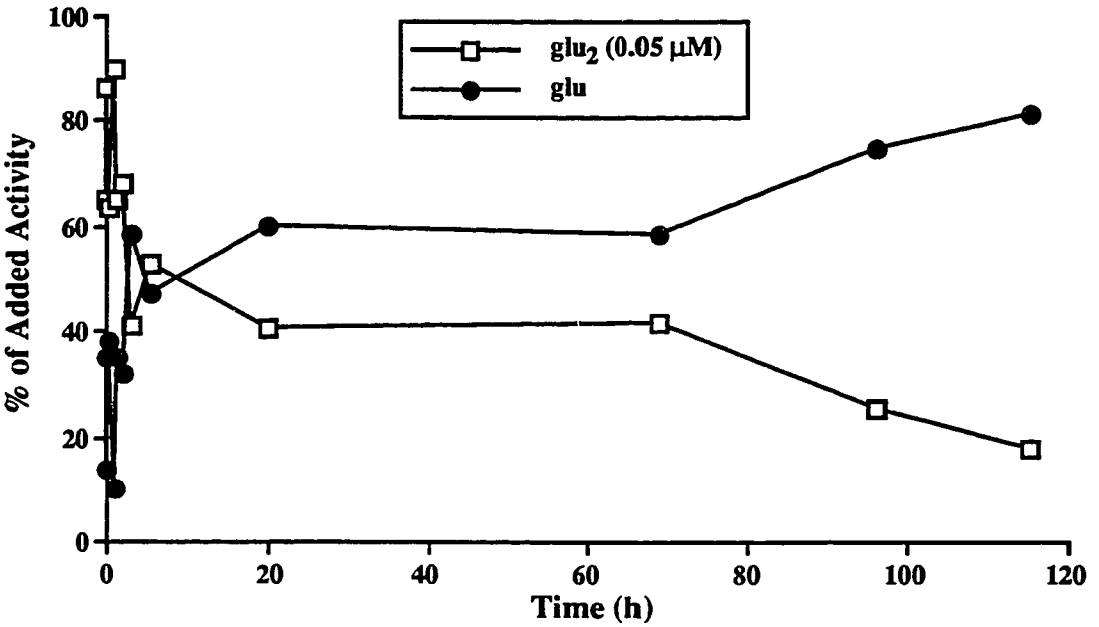


Figure 2.5. Decomposition of ^{3}H-Glu_2 in Skan Bay Pore Water (Long-Term Incubation). Concentrations indicated in parentheses are the initial dissolved peptide concentration in pore water before decomposition.

The % of ^{3}H-glu$_2$ hydrolysis at 0.05 μM varied randomly between 15 and 60% (33.7±18.2% on average) for times of less than 1 hour (Figure 2.5), even though the series of separate vials for each time point were taken from the same filtered pore water and prepared in the same way. Each vial was degassed for the same period of time, and the volume ratio of pore water and added solution was kept the same.

Figure 2.6 (a and b) shows the effect of peptide concentration on the hydrolysis of ^{3}H-glu$_2$ and ^{3}H-ala$_2$ in pore water for a 1 minute mixing time. The hydrolysis rates of both peptides increased with concentration. At each concentration, the hydrolysis rate of ^{3}H-glu$_2$ was greater than that of ^{3}H-ala$_2$, and the increase of ^{3}H-glu$_2$ hydrolysis with concentration was greater than that of ^{3}H-ala$_2$. The hydrolysis of ^{3}H-ala$_2$ reached saturation at 500 μM, but hydrolysis did not reach saturation for ^{3}H-glu$_2$ even at 45 mM which is near the maximum solubility of glu$_2$ in seawater.

Decomposition of ^{3}H-peptides in sediment One decomposition experiment was conducted in fresh sediments at a peptide concentration of 0.03 μM over a time interval of 48 hours. The decomposition was followed by measuring the products, ^{3}H-FAA and ^3H_2O, hydrolyzed and respired from added ^{3}H-peptides. The ^{3}H-FAA and ^3H_2O in pore water, added ^{3}H-peptide remaining in pore water, and ^{3}H-FAA and ^{3}H-peptide in HCl extracts were measured.

Figure 2.7 shows the hydrolysis of the ^{3}H-peptides vs. time. After 15 minutes, about 70% of the added ^{3}H-glu$_2$, 45% of ^{3}H-ala$_2$, 25% of ala$_3$ and 20% of ala$_6$ and lys$_2$ was hydrolyzed. The extent of ^{3}H-glu$_2$ hydrolysis remained unchanged after the first time point. The hydrolysis of ^{3}H-ala$_2$ reached a maximum of 60% after 5 hours and that of ^{3}H-lys$_2$ after 24 hours, while the hydrolysis of ^{3}H-ala$_3$ and ^{3}H-ala$_6$ increased gradually over time to about 90% after 48 hours. Figure 2.8 shows the respiration of ^{3}H-

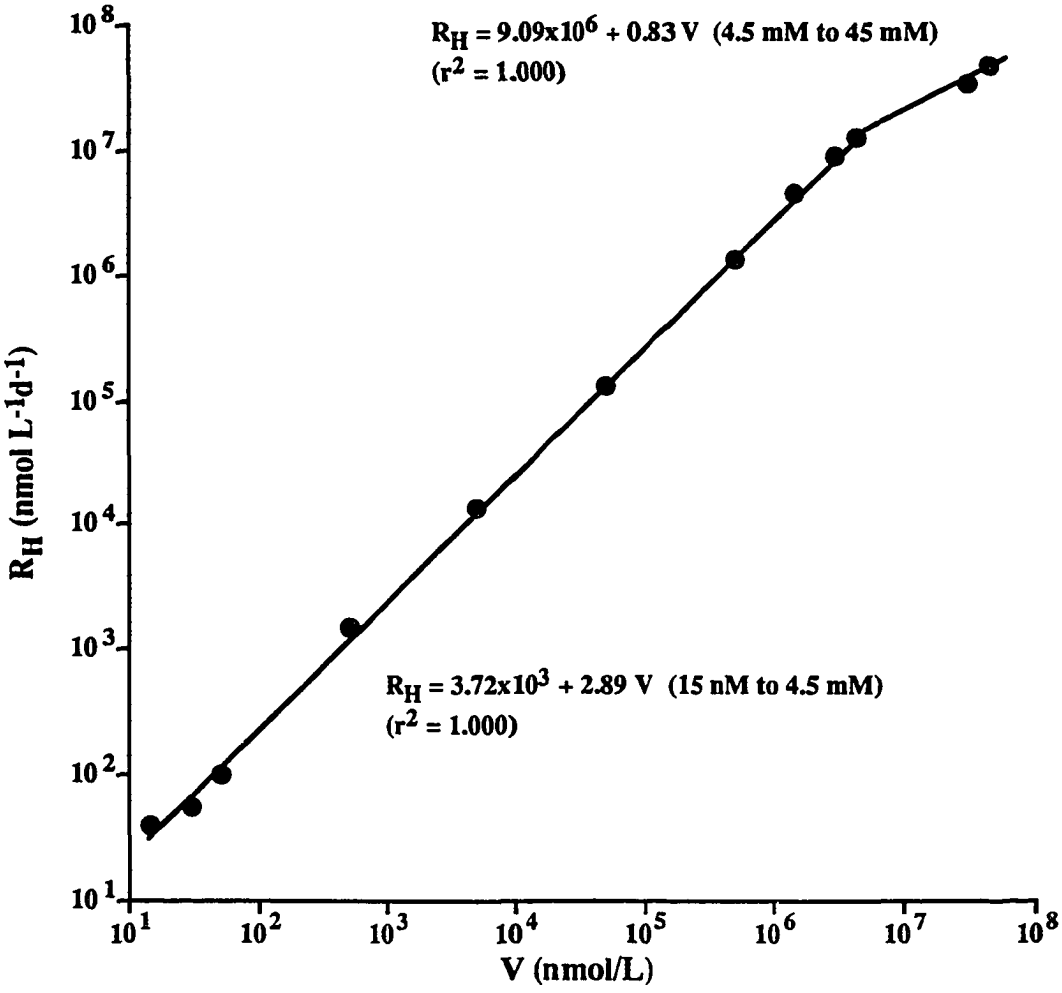


Figure 2.6a. Effect of Peptide Concentration on Hydrolysis of ^{3}H-Glu_2 in Skan Bay Pore Water. R_H is hydrolysis rate and V is concentration.

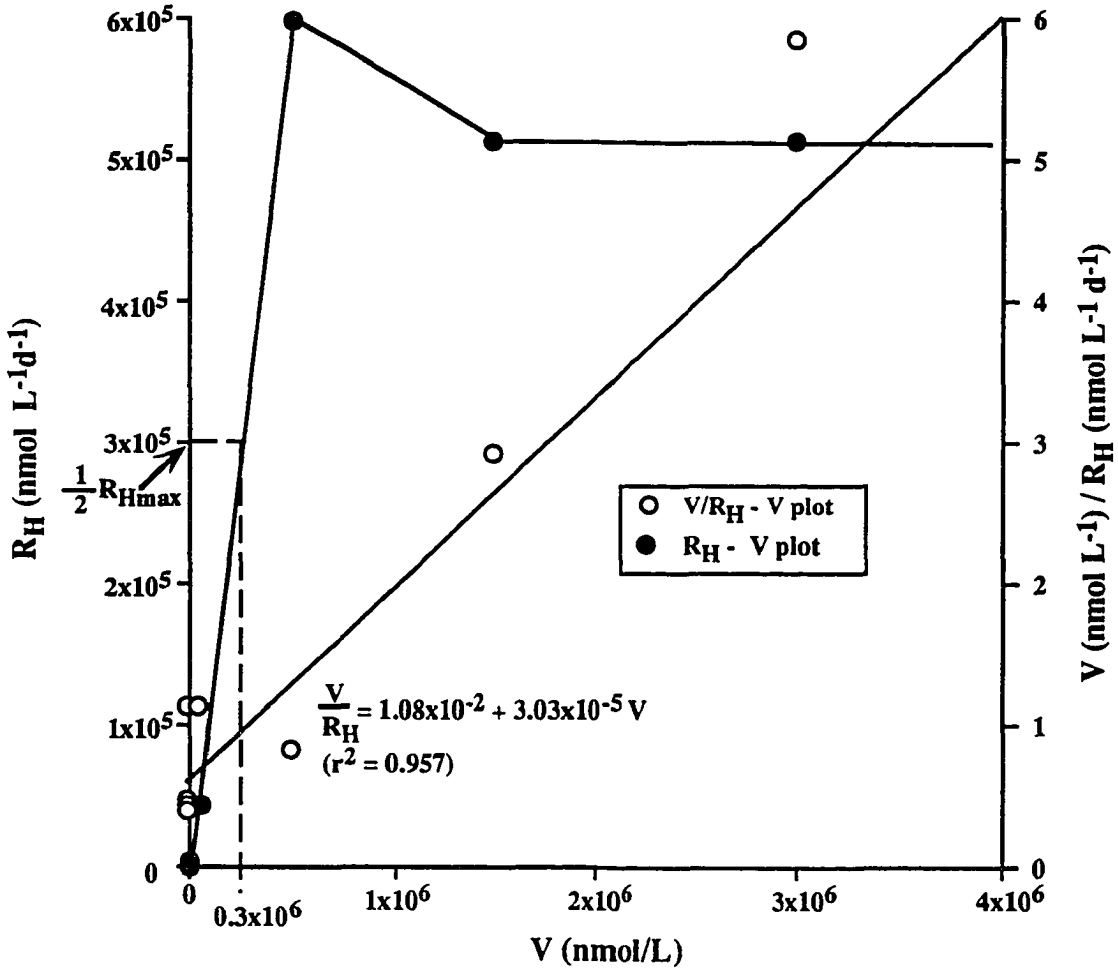


Figure 2.6b. Effect of Peptide Concentration on Hydrolysis of ^{3}H-Ala$_2$ in Skan Bay Pore Water. R_H is hydrolysis rate and V is concentration.

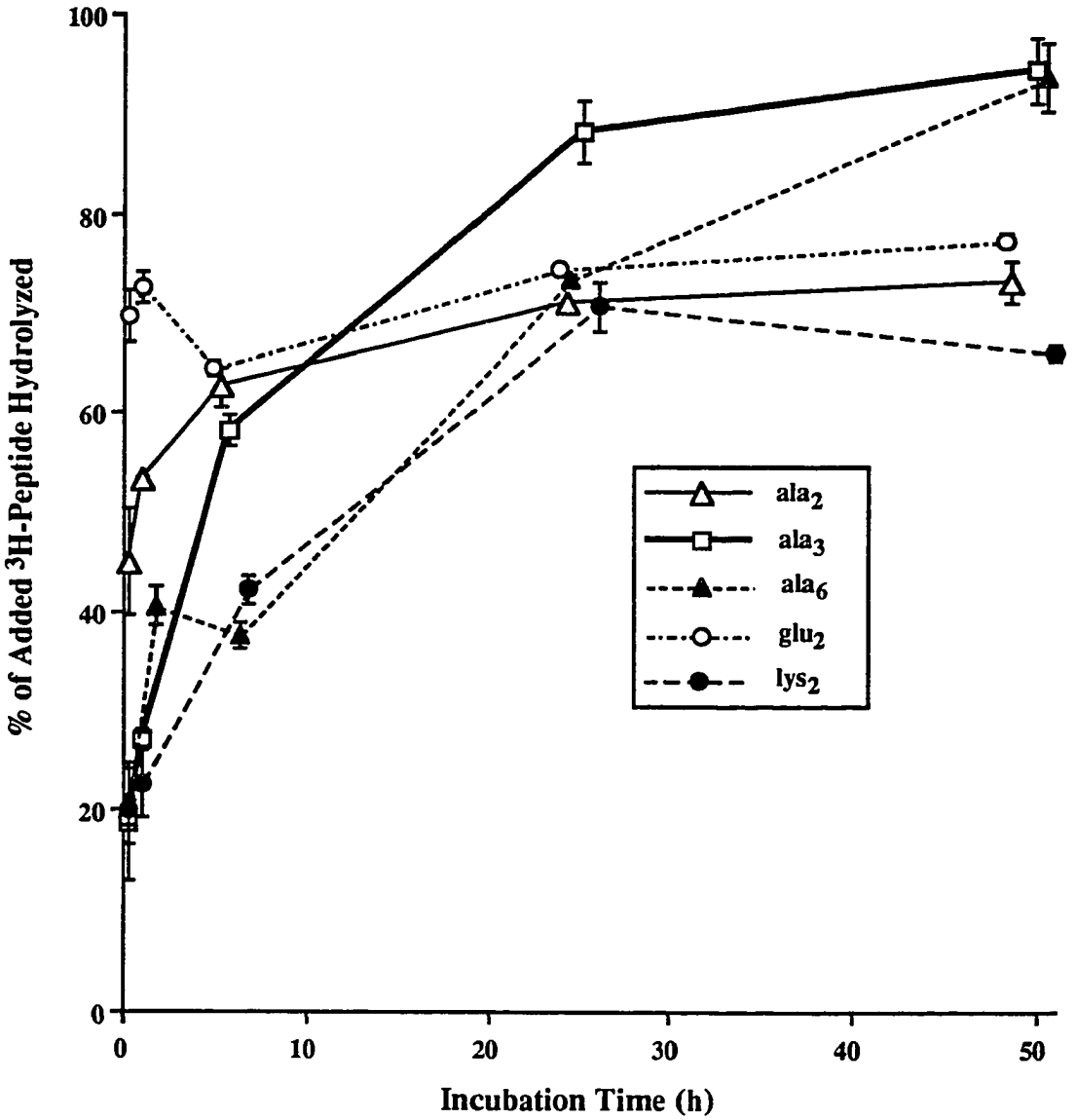


Figure 2.7. Hydrolysis of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Skan Bay Sediments.

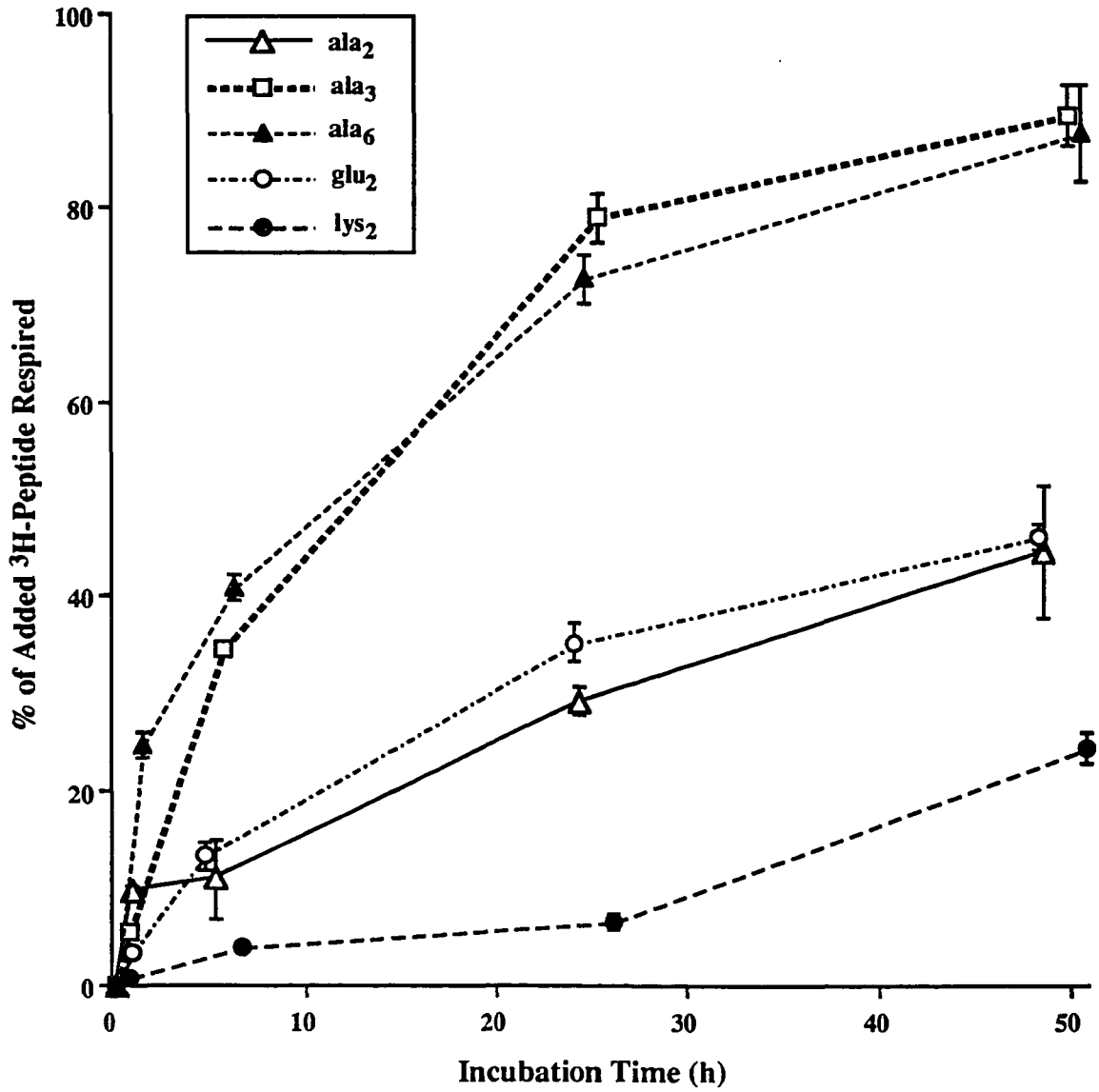


Figure 2.8. Respiration of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Skan Bay Sediments.

peptides. After 48 hours, up to 90% of added ^{3}H-ala$_3$ and ^{3}H-ala$_6$, about 45% of ^{3}H-glu$_2$ and ^{3}H-ala$_2$, and 20% of ^{3}H-lys$_2$ were respired.

The fate of ^{3}H-peptides added to sediments is shown in Figure 2.9. The amount of ^{3}H-FAA in pore water (from both the added solution and hydrolysis of ^{3}H-peptides) decreased substantially by respiration and adsorption after 15 minutes. After 24 hours, there was no measurable ^{3}H-FAA in pore water. ^{3}H-peptide in pore water decreased substantially after 1 hour and it was less than 10% of the added amount after 48 hours. For ^{3}H-ala$_3$ and ^{3}H-ala$_6$, no ^{3}H-FAA was extracted in the HCl solution. However, about 5% of added ^{3}H-activity was extracted as ^{3}H-FAA for ^{3}H-ala$_2$. For ^{3}H-lys$_2$, the ^{3}H-lysine extracted increased from 5% of the added activity at 15 minutes to about 15% after 5 hours, then it remained relatively constant to 48 hours. For ^{3}H-glu$_2$, HCl extractable ^{3}H-glutamic acid increased from 5% of total activity at 15 minutes to 30% after 1 hour, and then gradually decreased to 5%. There was basically no ^{3}H-ala$_2$, ala$_3$ and ala$_6$ extracted in the HCl solution. The pattern of change in the acid-extractable fraction of ^{3}H-lys$_2$ and ^{3}H-glu$_2$ over time was similar to that of ^{3}H-glutamic acid. The maximum amount of ^{3}H-lys$_2$ in the acid extract (18%) appeared at 5 hours and that of ^{3}H-glu$_2$ (20%) at 1 hour.

At 15 to 18 minutes, the proportion of added peptide hydrolyzed generally decreased or remained about the same with increasing added peptide concentration. However, the hydrolysis rates, calculated in units of nmol per cm^3 dry sediment per day, increased with concentration. In the concentration range from 0.01 μM to 3.33 μM, the relative hydrolysis rates of the five ^{3}H-peptides were: ^{3}H-glu$_2$ > ^{3}H-ala$_2$ > ^{3}H-ala$_6$ ≥ ^{3}H-ala$_3$ > ^{3}H-lys$_2$ (Figure 2.10).

The hydrolysis rates of ^{3}H-peptides in fresh and stored sediments were different, except for those of ^{3}H-glu$_2$. In the concentration range from 0.03 μM to 3.33 μM, the

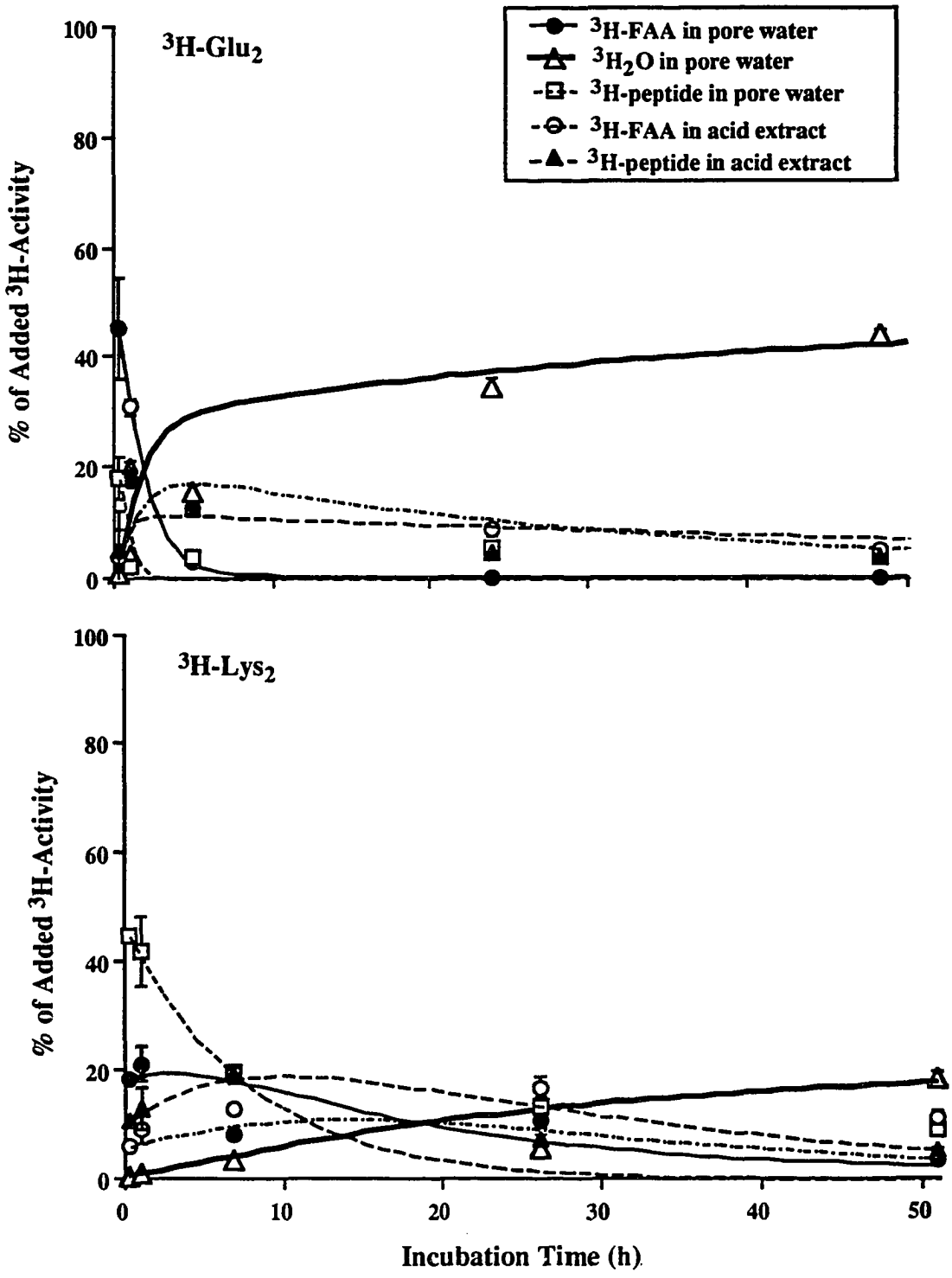


Figure 2.9. Decomposition of ^{3}H-Peptides in Skan Bay Sediments. Lines drawn were calculated using a model described in the text and parameters given in Table 2.2.

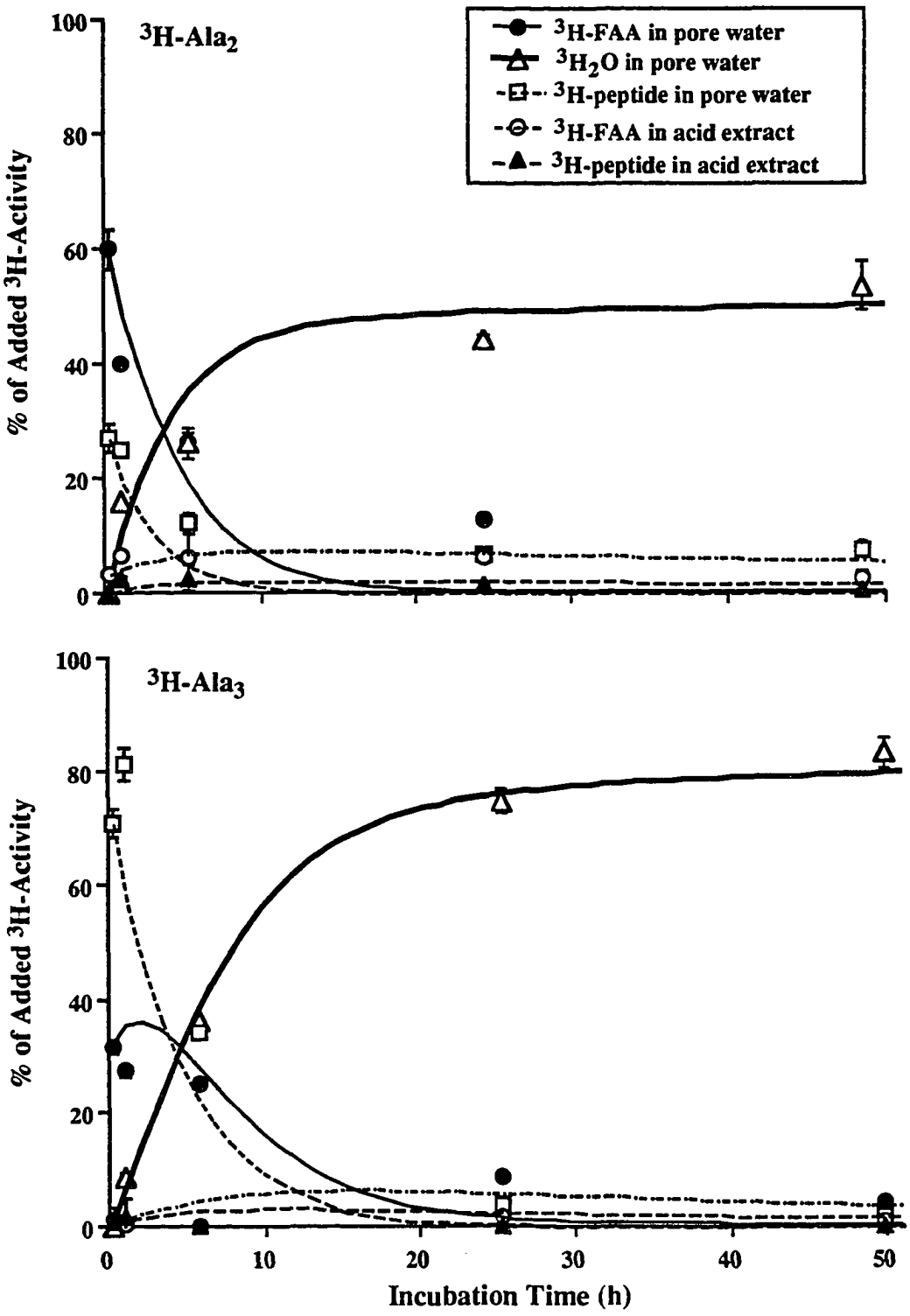


Figure 2.9. (continued)

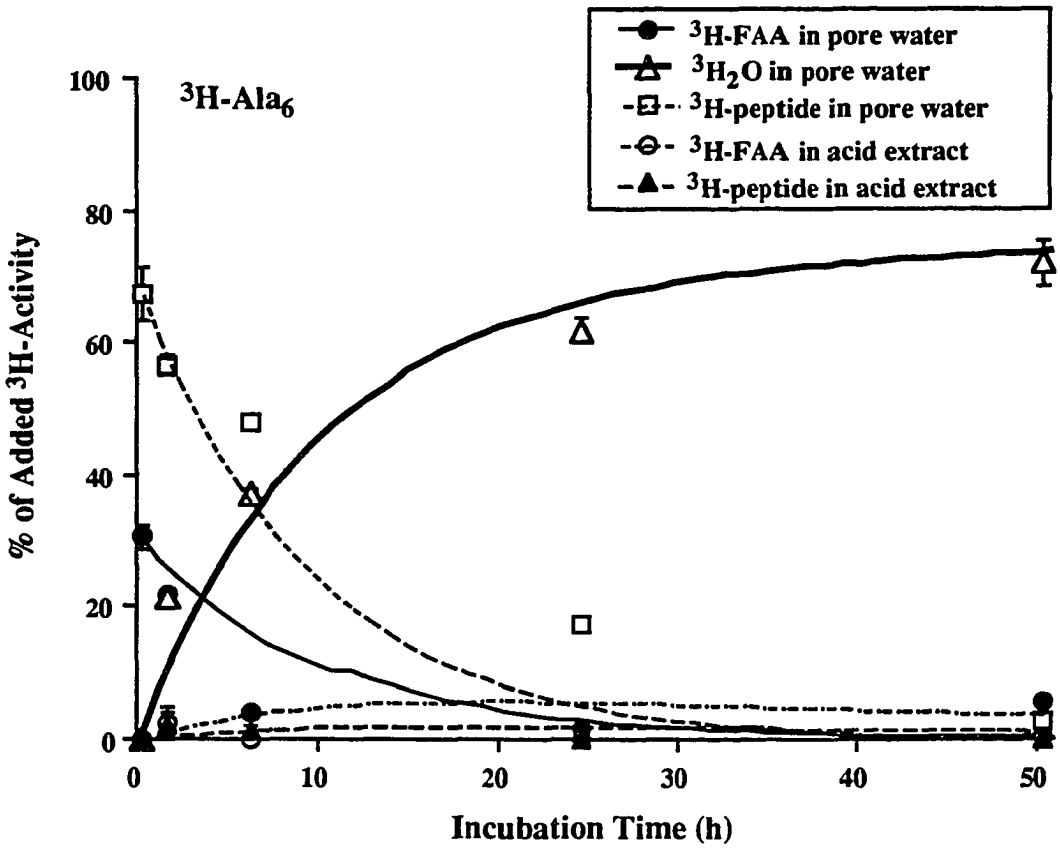


Figure 2.9. (continued)

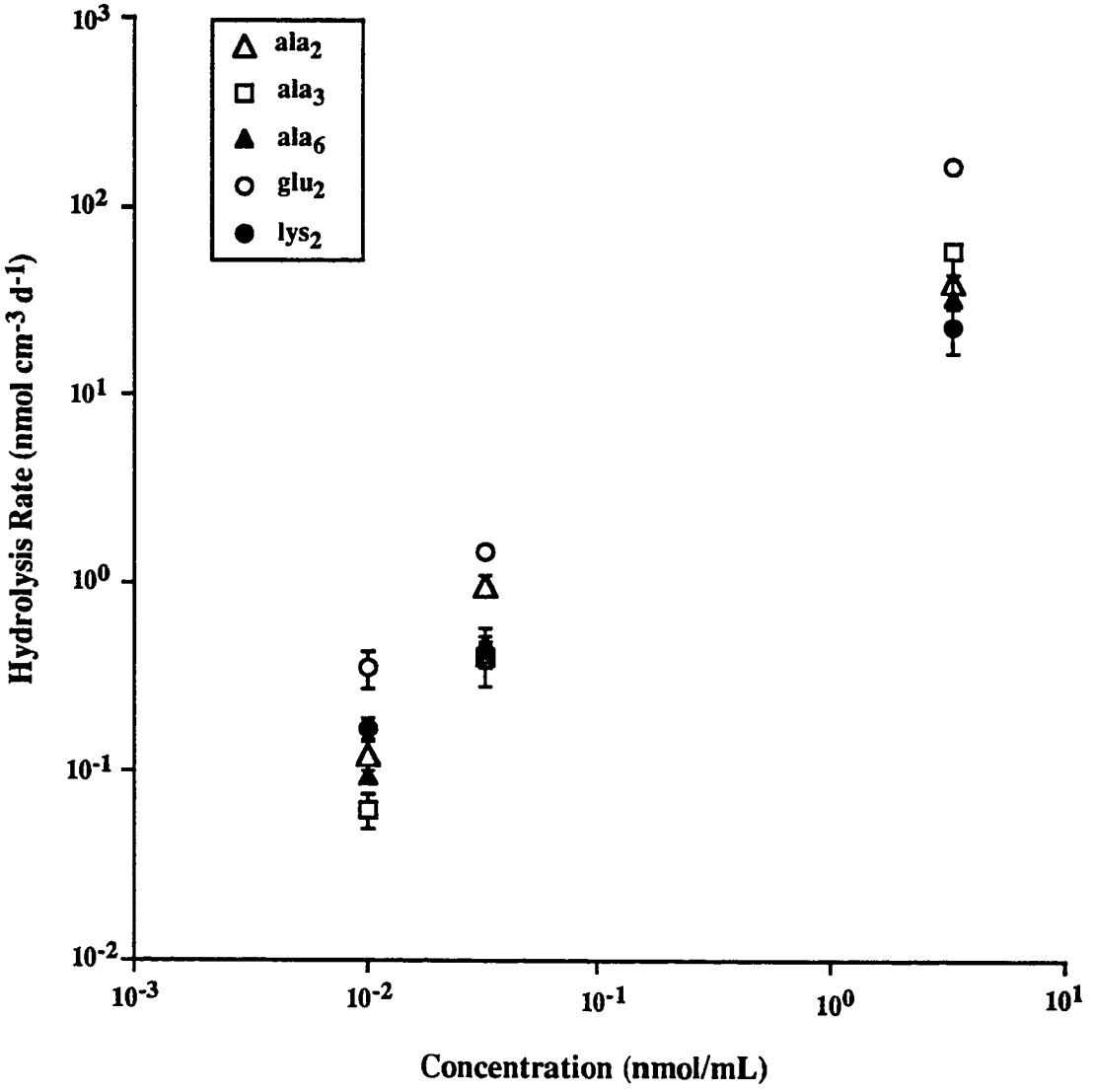


Figure 2.10. Effect of Concentration on Hydrolysis of ^{3}H-Peptides in Skan Bay Sediments

hydrolysis rates in stored sediments were 4 to 20 times greater than those in fresh sediments (Figure 2.11). However, respiration in both fresh and stored sediments was less than 5% of added activity (APPENDIX III, Table A.3).

As the proportion of ^{3}H-glu$_2$ hydrolyzed at 0.03 μM concentration in filtered pore water (63%) was similar to that in sediment (70%), extracellular enzymes in pore water could have been responsible for the rapid hydrolysis in sediment. To test this idea, filtered (0.2 μm Nuclepore) seawater was used to rinse the sediment five times to remove and dilute the original pore water, but the hydrolysis of ^{3}H-glu$_2$ in rinsed and untreated sediments were similar; 71% and 62% was hydrolyzed after 15 minutes in the rinsed and untreated sediments, respectively. Thus, enzymes were mainly associated with sediment particles.

Killed controls in sediment Neither chilling nor heat treatment stopped hydrolysis completely. Formalin reacted rapidly with peptides (APPENDIX IV, Figure A.2). The formalin reaction and hydrolysis removed most of the peptides in the dissolved pool (APPENDIX IV, Figures A.3a, b, c, d, and e). Autoclaving, followed by seawater rinse, was found relatively effective in eliminating peptide hydrolysis.

Only autoclaving for 2 hours nearly stopped peptide hydrolysis (Figures 2.12, 2.13). A seawater rinse of autoclaved sediment was adopted for two reasons: pore waters from autoclaved sediment were dark orange, which caused quenching during scintillation counting, and had elevated amino acid concentrations that affected amino acid adsorption. Rinsing eliminated these problems. Figure 2.12 also shows the results of the other treatments on the hydrolysis of ^{3}H-glu$_2$. Compared with untreated sediment, chilling (-1.5°C, 2 hours), heat treatment (69°C and 85°C, 3 hours), and autoclaving for 1 hour did not slow hydrolysis. At 0.03 μM peptide concentration, autoclaving for 2 hours

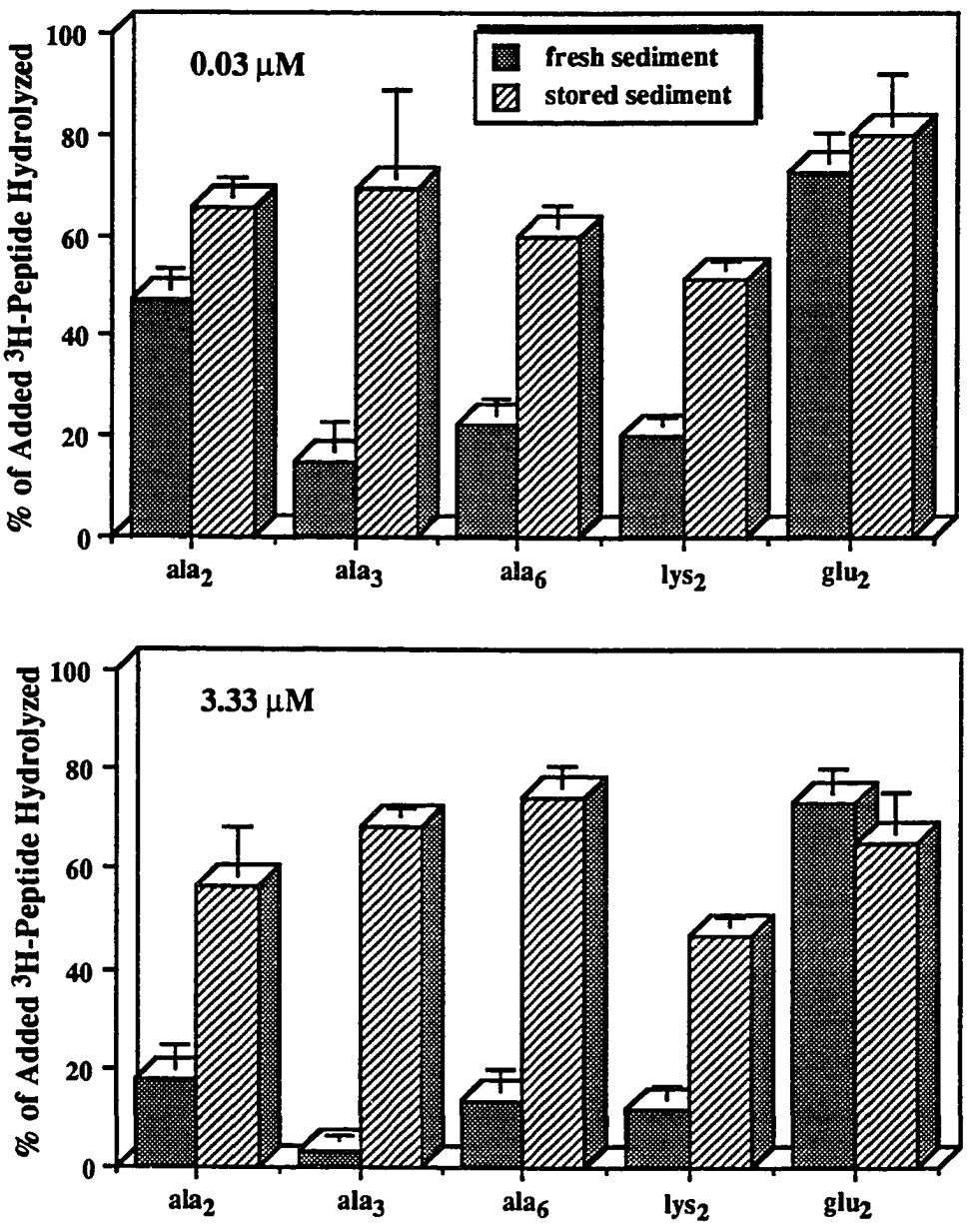


Figure 2.11. Hydrolysis of ^{3}H-Peptides in Skan Bay Sediments (18 Minute Incubation). Concentrations are the initial dissolved peptide concentration in pore water, before adsorption.

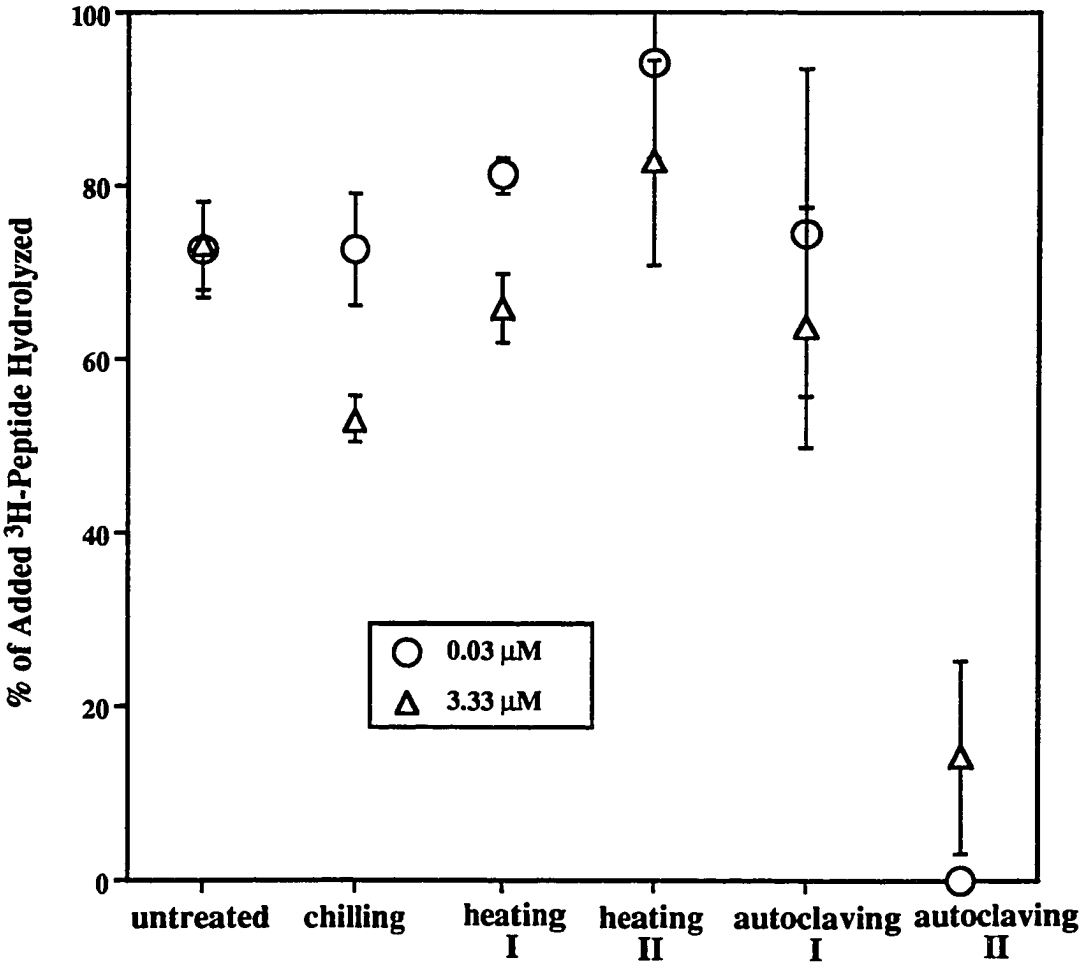


Figure 2.12. Hydrolysis of ^{3}H-Glu_2 in Skan Bay Sediments. Incubation time = 18 minutes; chilling = -1.5°C, 3 hours; heating I = 69°C, 3 hours; heating II = 85°C, 3 hours; autoclaving I =15p.s.i, 120°C, 1 hours; autoclaving II = 15 p.s.i, 120°C, 2 hours. Concentrations indicated are the initial dissolved peptide concentration in pore water before adsorption.

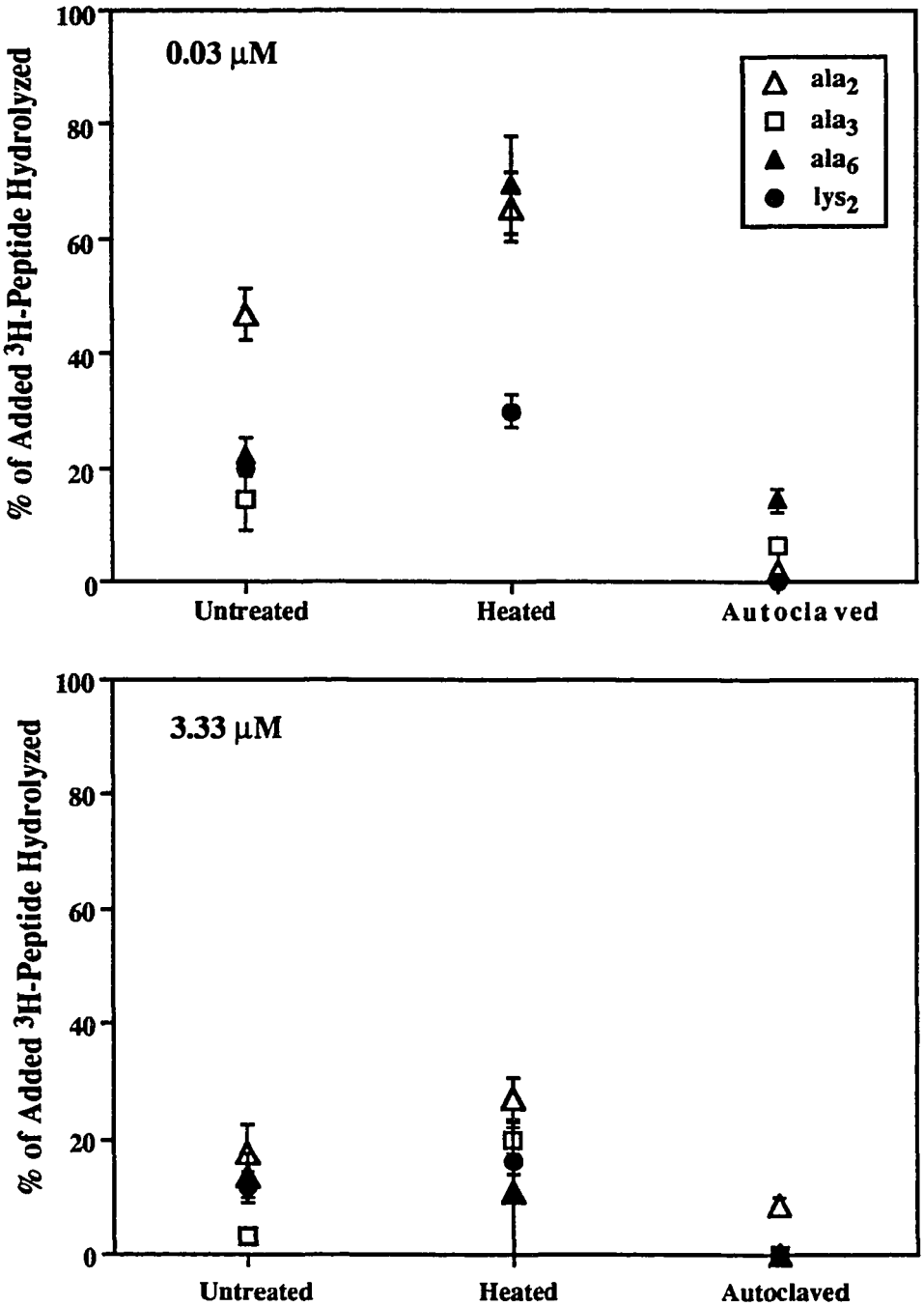


Figure 2.13. Hydrolysis of ^{3}H-Peptides in Skan Bay Sediments. Incubation Time = 18 minutes. Concentrations are the initial dissolved peptide concentration in pore water before adsorption. Heated = 85°C, 3 hours. Autoclaved = 15 p.s.i, 120°C, 2 hours.

eliminated hydrolysis and at 3.33 μM, only a small amount of enzymatic activity remained.

Figure 2.13 shows the results of heat and autoclave treatments of sediments on the decomposition of ^{3}H-ala$_2$, ^{3}H-ala$_3$, ^{3}H-ala$_6$ and ^{3}H-lys$_2$. Heat treatment (85°C, 3 hours) usually increased hydrolysis rates. Autoclaving stopped most of the enzymatic activity, except that 10 to 16% of the added ^{3}H-alanyl peptides were hydrolyzed in two of the experiments. The autoclaved sediment was kept at 2 to 3°C for 12 hours before the decomposition experiments were conducted.

Autoclaved sediments retained a low level of enzymatic activity (Figures 2.14, 2.15). Less than 5% of the added ^{3}H-ala$_2$, ala$_3$, glu$_2$ and lys$_2$ was hydrolyzed within 18 minutes (Figure 2.14). The hydrolysis of ^{3}H-ala$_2$ and lys$_2$ did not increase with time, but hydrolysis of ^{3}H-glu$_2$, ala$_3$ and ala$_6$ increased for the first 5 hours. The % of ^{3}H-peptides hydrolyzed was: lys$_2$ ≅ ala$_2$ (5%) < glu$_2$ (20%) < ala$_3$ (30%) < ala$_6$ (60%). The pattern of ^{3}H-peptide respiration over time was similar to that of hydrolysis. There was no respiration at the first time point (Figure 2.15). Over 48 hours, the respiration of ^{3}H-ala$_2$, glu$_2$ and lys$_2$ was less than 3%. Respiration was 10 to 15% for ^{3}H-ala$_3$, and 40 to 45% for ^{3}H-ala$_6$ after 5 hours. This production of ^3H_2O could not have been due to simple solvent exchange of hydrogen, because it did not occur in pore water alone.

However, the decomposition of added ^{3}H-peptides and ^{3}H-FAA in autoclaved sediments was much slower than in untreated sediments. After 48 hours, both ^{3}H-peptide and ^{3}H-FAA remained in pore water of autoclaved sediments. The relative amount of ^{3}H-peptide and ^{3}H-FAA in pore water was still similar to that in added solution: 50 to 70% of the added ^{3}H-activity was ^{3}H-peptide and 20 to 30% was ^{3}H-FAA. The amount of ^{3}H-FAA and ^{3}H-peptide in the acid extract was 5 to 10% of the

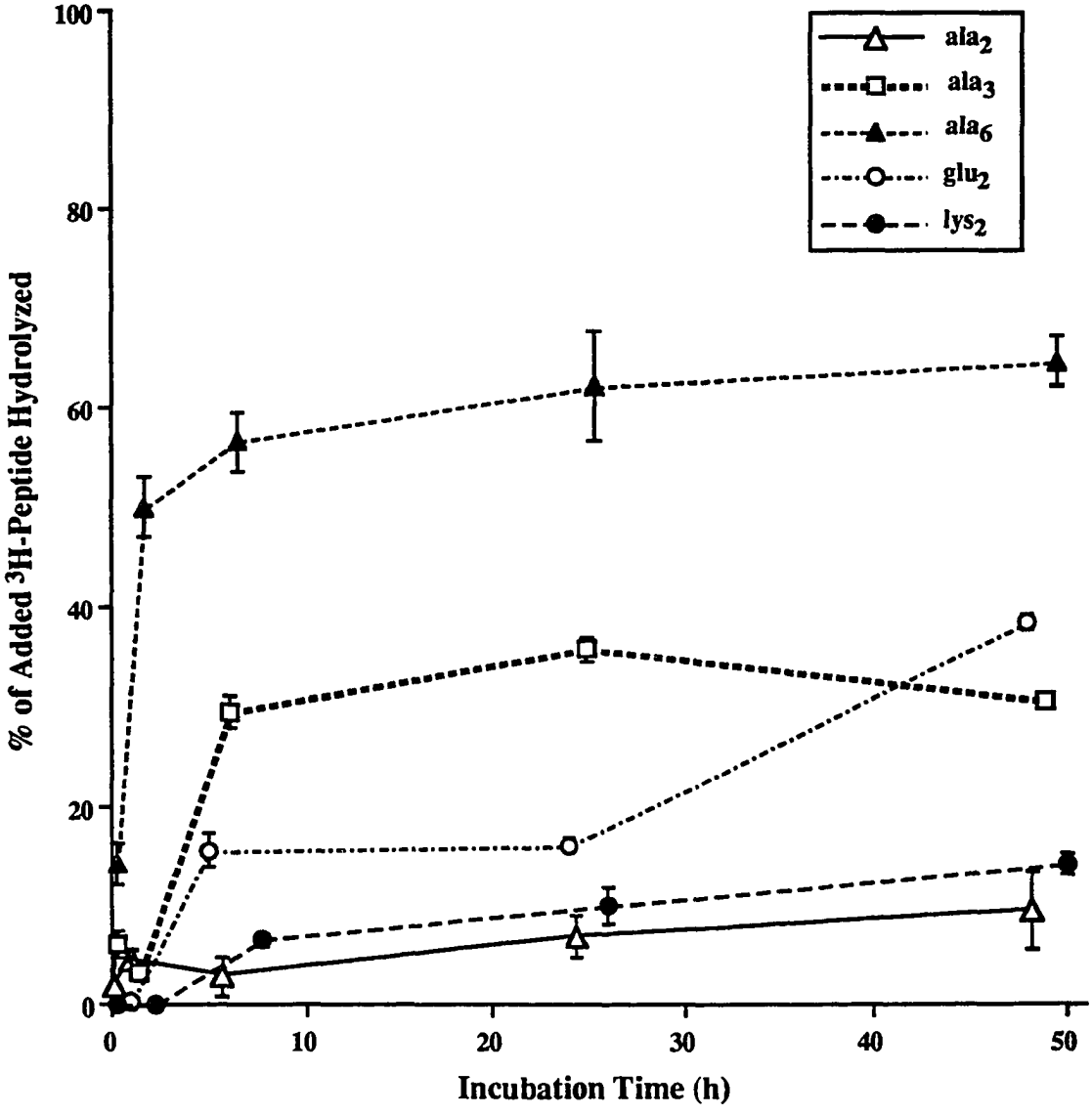


Figure 2.14. Hydrolysis of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Autoclaved Skan Bay Sediments.

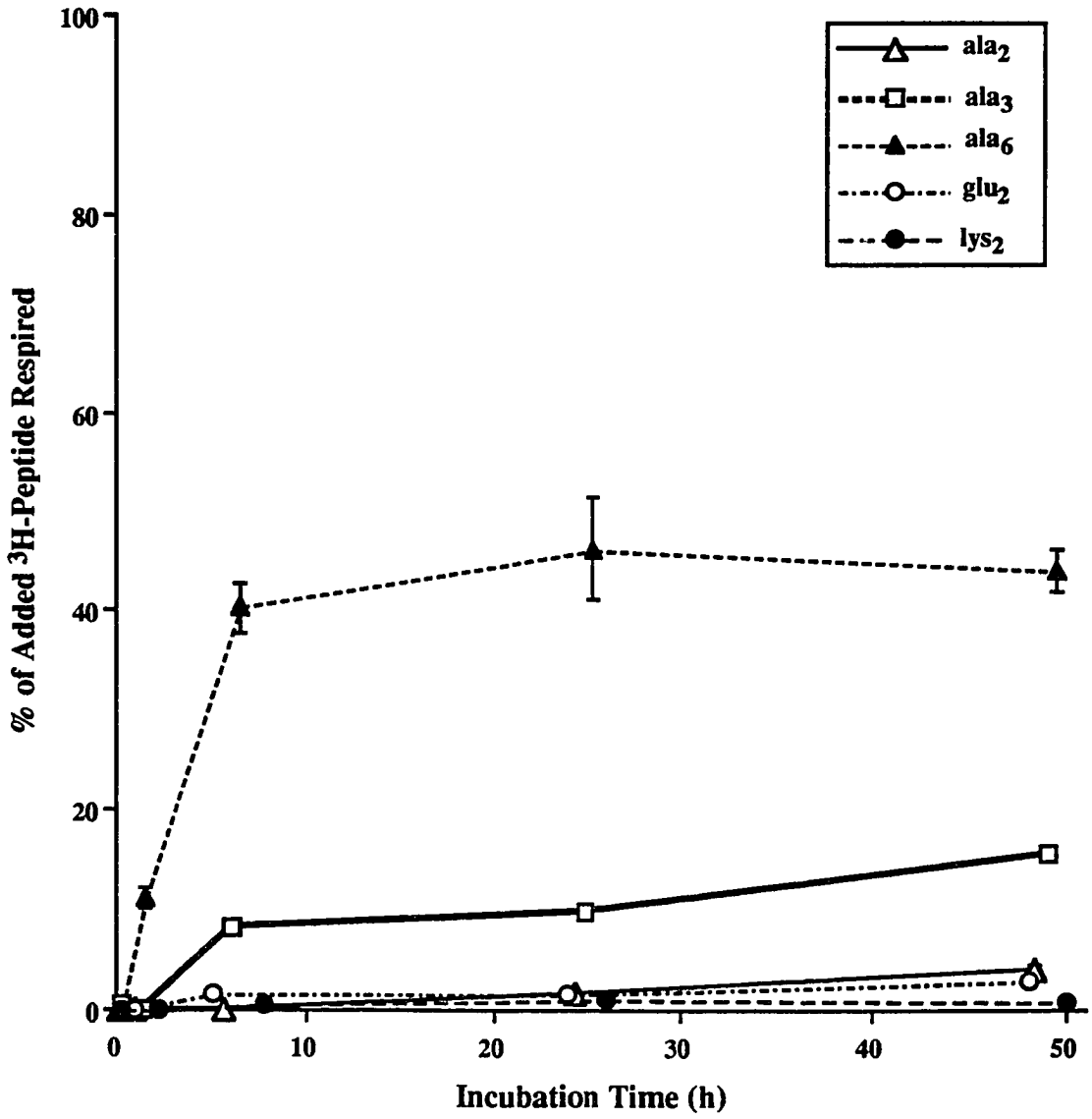


Figure 2.15. Respiration of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Autoclaved Skan Bay Sediments.

added ^3H-activity. The ^3H_2O in pore water was likewise much less than that produced due to respiration in untreated sediments (Figure 2.8).

Adsorption of peptides in sediment Figure 2.16 compares the adsorption of peptides in autoclaved and untreated sediments within 15 to 18 minutes of peptide addition. With both fresh and stored sediments, the adsorption of ^3H-peptide during 15 to 18 minutes incubation in untreated sediments was statistically (F test with $\alpha = 0.05$, Mendenhall 1987; APPENDIX III, Table A.6) similar to that in autoclaved sediments, except that adsorption of ^3H-glu_2 and ala_3 at 0.03 μM and ^3H-ala_2 at 3.33 μM was greater in untreated sediment. For most of the experiments, especially in autoclaved sediments, adsorption of ^3H-lys_2 was greater than that of other peptides.

For ^3H-peptides in autoclaved sediment, concentration had little effect on adsorption (Figure 2.17). Over the concentration range of 0.01 μM to 333 μM, the adsorption of ^3H-lys_2 did not vary with concentration and was greater than that of the other peptides, although the difference was not significant. The adsorption of ^3H-ala_2, ^3H-ala_3, ^3H-ala_6 and ^3H-glu_2 was similar except that the adsorption of ^3H-ala_2 doubled when the concentration increased to 3.33 μM, and then stayed constant in more concentrated solutions.

The effects of time on adsorption were investigated. In untreated sediments, there was some increase in adsorption of ^3H-peptides with time (Figure 2.18). Before 24 hours, the adsorption of ^3H-peptides was: $lys_2 \approx glu_2 > ala_2$; after 24 hours, the adsorption became: $lys_2 \approx glu_2 \approx ala_2$. In autoclaved sediments, the adsorption of ^3H-lys_2 and its change with time (Figure 2.19) were similar to those in untreated sediments (Figure 2.18), but the adsorption of ^3H-ala_2 and ^3H-glu_2 changed little in 48 hours. The relative adsorption of ^3H-peptides in autoclaved sediment was: $lys_2 > ala_2 > glu_2$. At

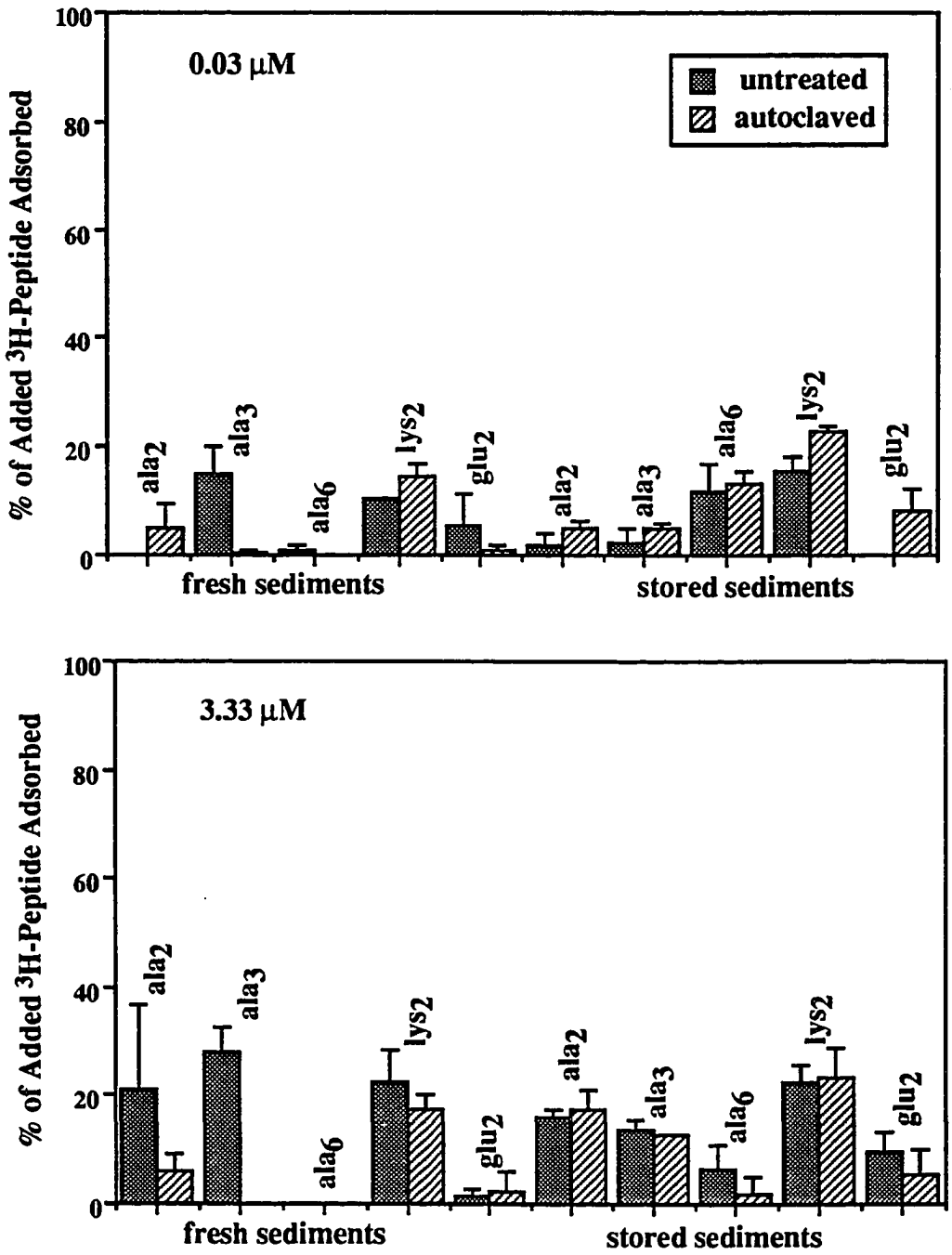


Figure 2.16. Adsorption of ^{3}H-Peptides by Untreated and Autoclaved Skan Bay Sediments. Concentrations are the initial dissolved peptide concentration in pore water before adsorption.

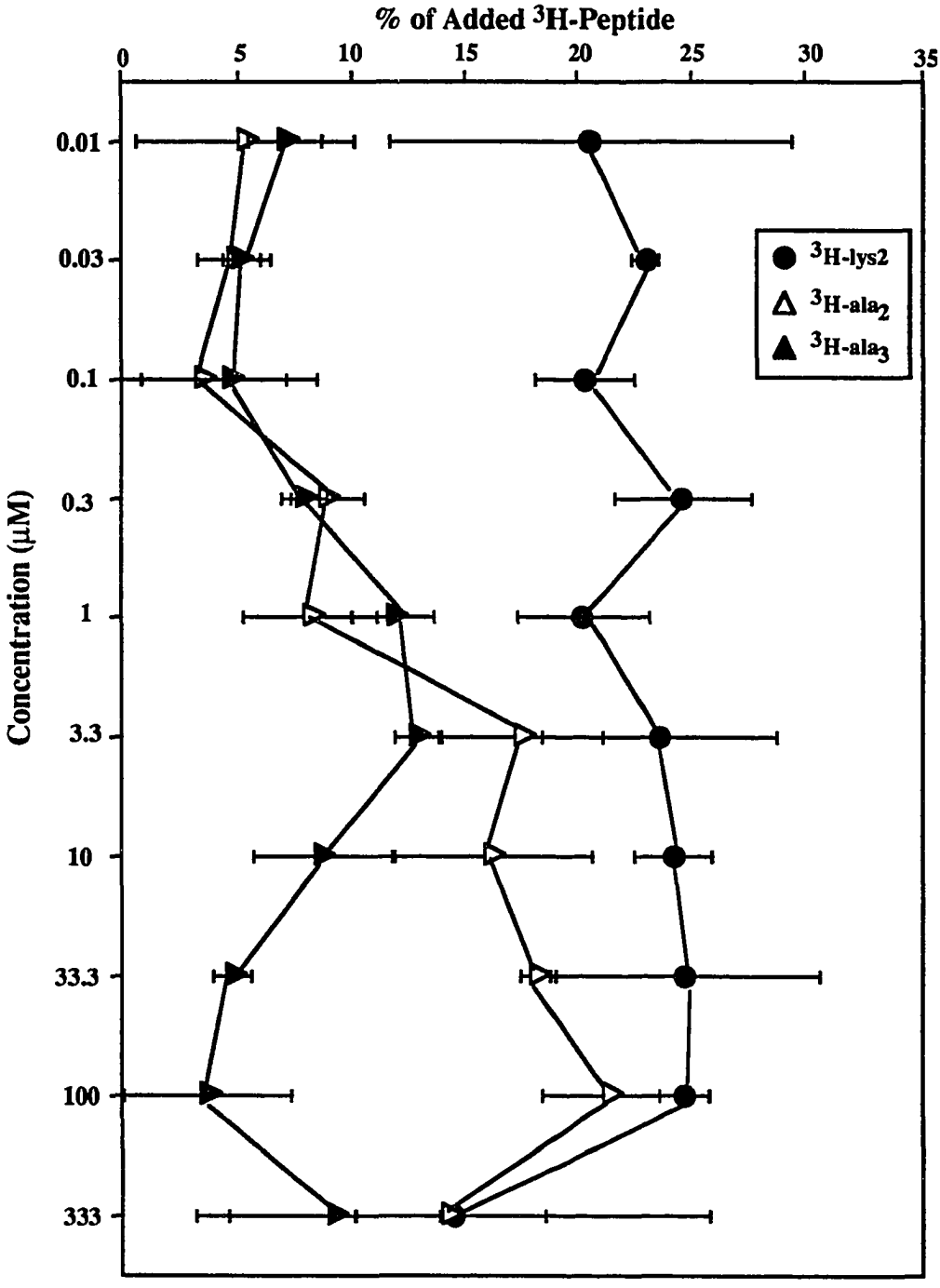


Figure 2.17. Adsorption of ^{3}H-Peptides by Skan Bay Sediments (Stored and Autoclaved).

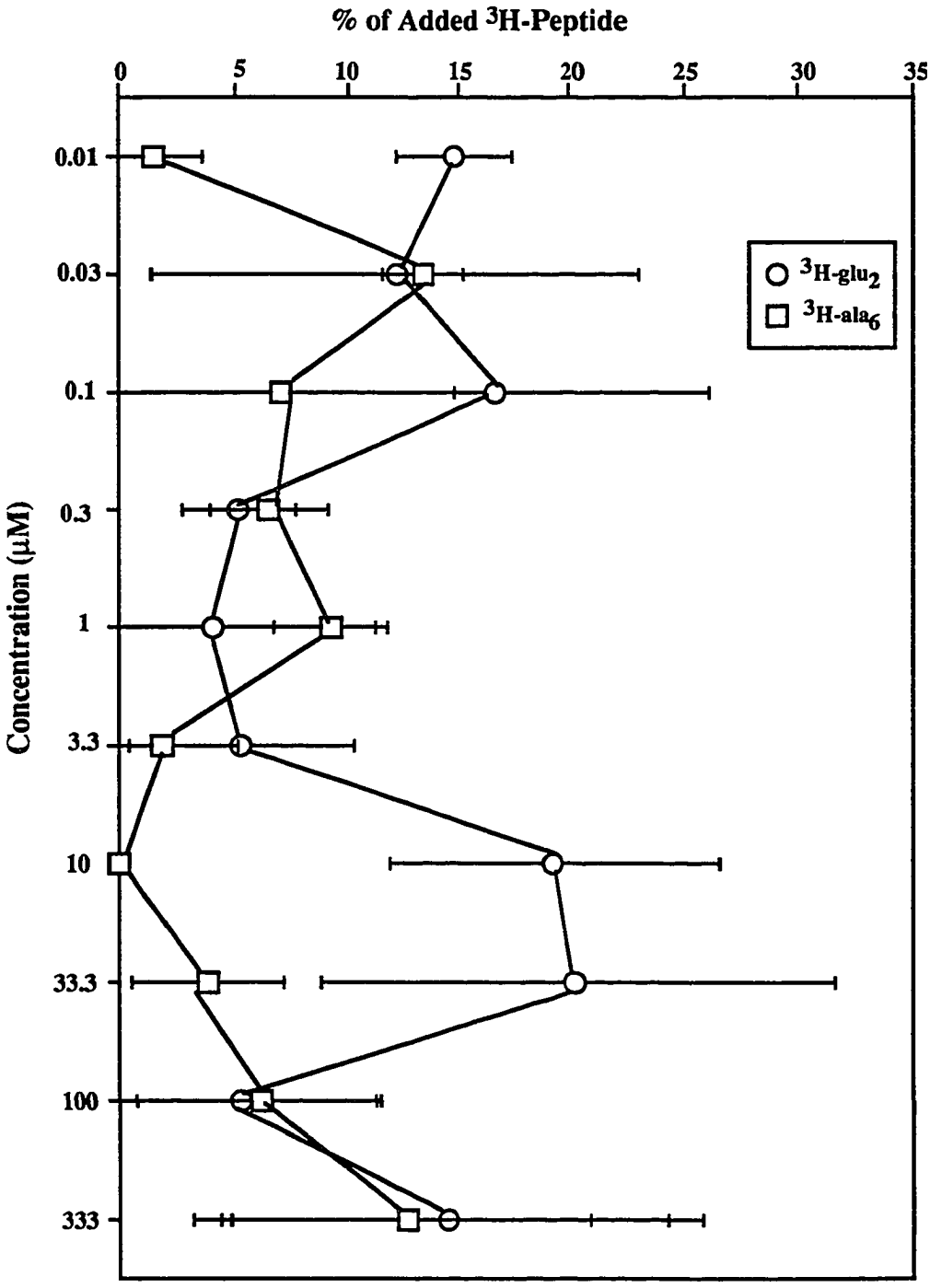


Figure 2.17. (continued)

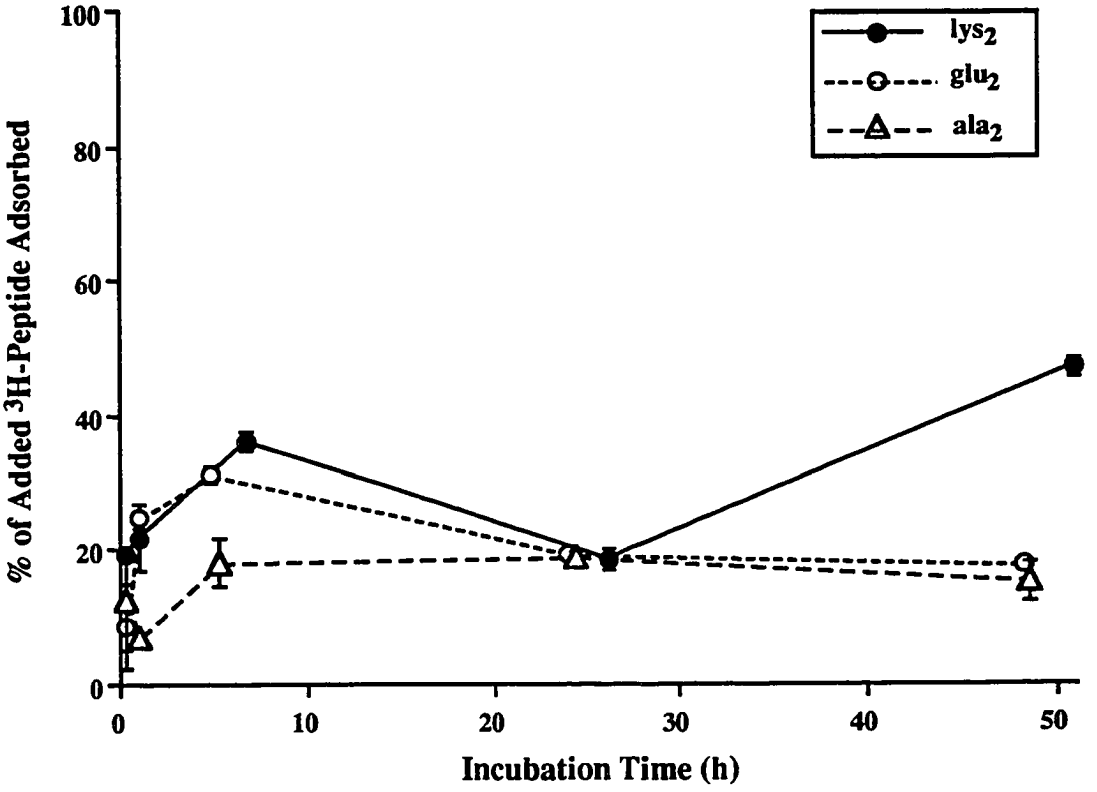


Figure 2.18. Effect of Incubation Time on Adsorption of ^{3}H-Peptides (at 0.03 μM Initial Concentration) by Skan Bay Sediments.

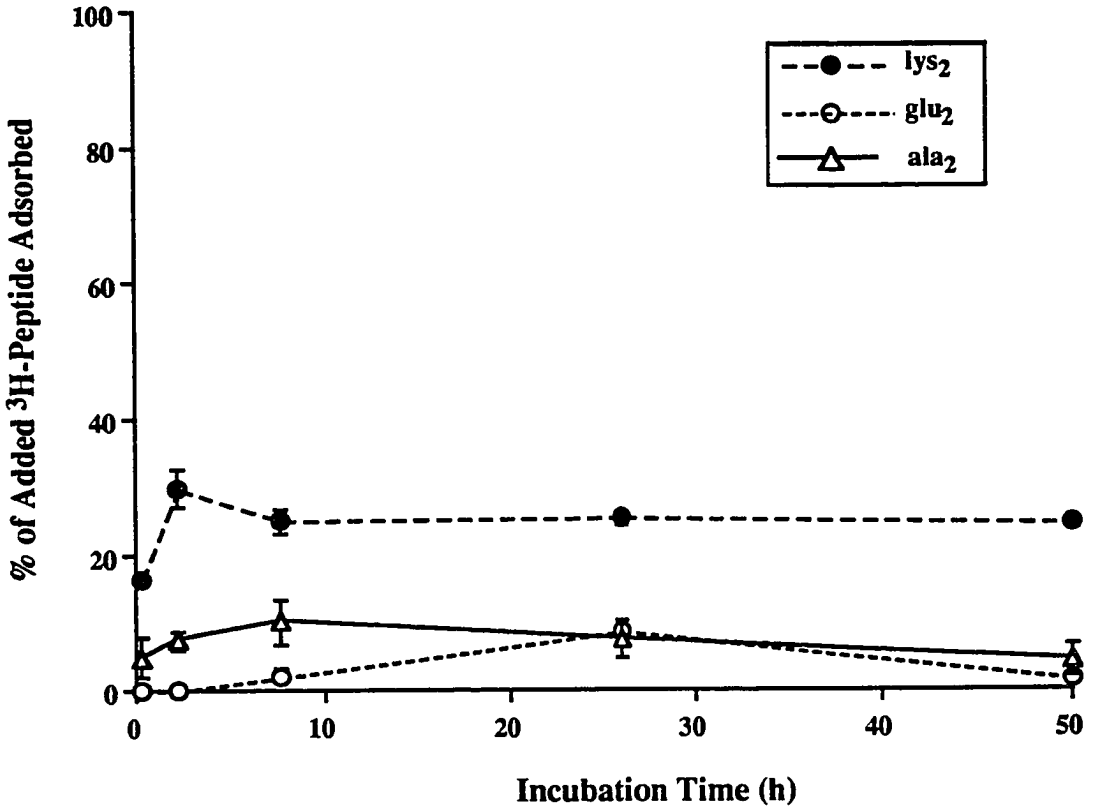


Figure 2.19. Effect of Incubation Time on Adsorption of ^{3}H-Peptides (at 0.03 μM Initial Concentration) by Autoclaved Skan Bay Sediments.

each time point, the peptide adsorption in autoclaved sediment was greater than that in untreated sediment.

Exchange and extraction of adsorbed ^{3}H-peptides from sediment In untreated sediments, the CsCl solution removed more ^{3}H-ala$_2$, ^{3}H-ala$_3$ and ^{3}H-lys$_2$ than seawater (Figures 2.20 and 2.21). This was also true in autoclaved sediments (Figures 2.22 and 2.23). More ^{3}H-peptides adsorbed at low concentration (e.g., 0.01 μM) were exchanged than those adsorbed at high concentration (e.g., 3.33 μM or 0.3 mM). The exchange solutions did not completely remove adsorbed ^{3}H-peptides.

From 0.01 μM to 0.3 mM peptide concentration, more adsorbed ^{3}H-peptides were extracted with 0.5 N HCl solution than with seawater, CsCl and NaAc (Figures 2.21, 2.22 and 2.23). However, HCl did not completely extract the adsorbed ^{3}H-peptides, except for ^{3}H-glu$_2$ adsorbed at 3.33 μM and 0.3 mM in autoclaved sediments. The NaOH solution was not effective in extracting ^{3}H-peptides (Figure 2.23).

In untreated sediment, the longer the incubation time, the less the adsorbed peptides were extracted with acid (Figure 2.24). Since ^{3}H-ala$_3$ and ^{3}H-ala$_6$ were not adsorbed by the sediments, the extraction of these two peptides is not shown. Apparent zero extraction of ^{3}H-ala$_2$ at 15 minutes probably is due to the large experimental error resulting from the small adsorption. Extractability of lys$_2$ and glu$_2$ decreases less than that of ala$_2$. In autoclaved sediment, the acid extractability of the adsorbed ^{3}H-ala$_2$ decreased over time (Figure 2.25), but not as much as in untreated sediments (Figure 2.24). The amount of adsorbed ^{3}H-lys$_2$ extracted did not change over time: 80 to 90% of the adsorbed ^{3}H-lys$_2$ was extracted by 0.5 N HCl. About 5 to 10% of the added ^{3}H-peptides, which could not be recovered by 0.5 N HCl extraction, were completely recovered by hydrolysis in hot 6N HCl solution (APPENDIX III, Table A.5). Adsorbed glu$_2$ was not extracted in four of the five experiments.

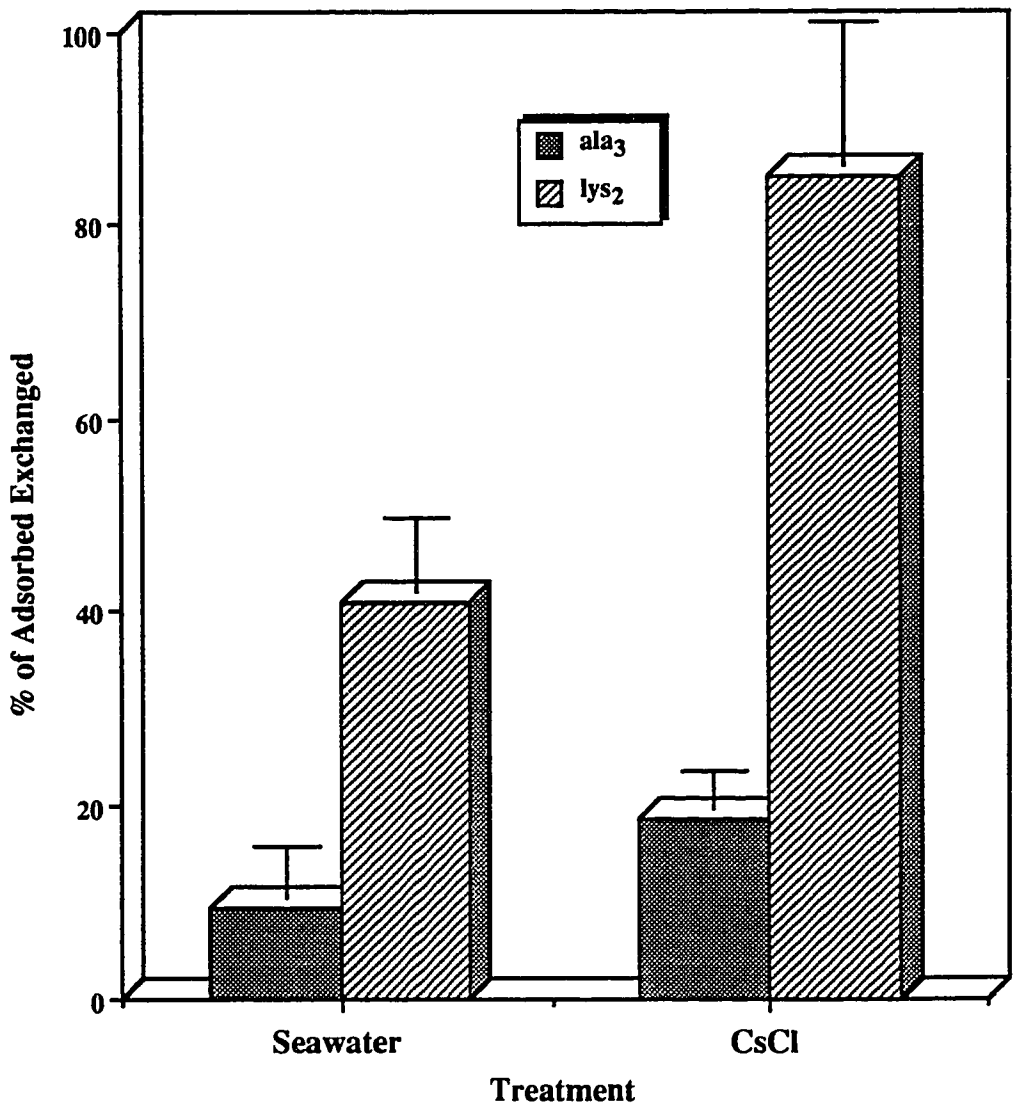


Figure 2.20. Exchange of Adsorbed ^{3}H-Peptides (at 0.01 μM Initial Concentration) from Skan Bay Sediments.

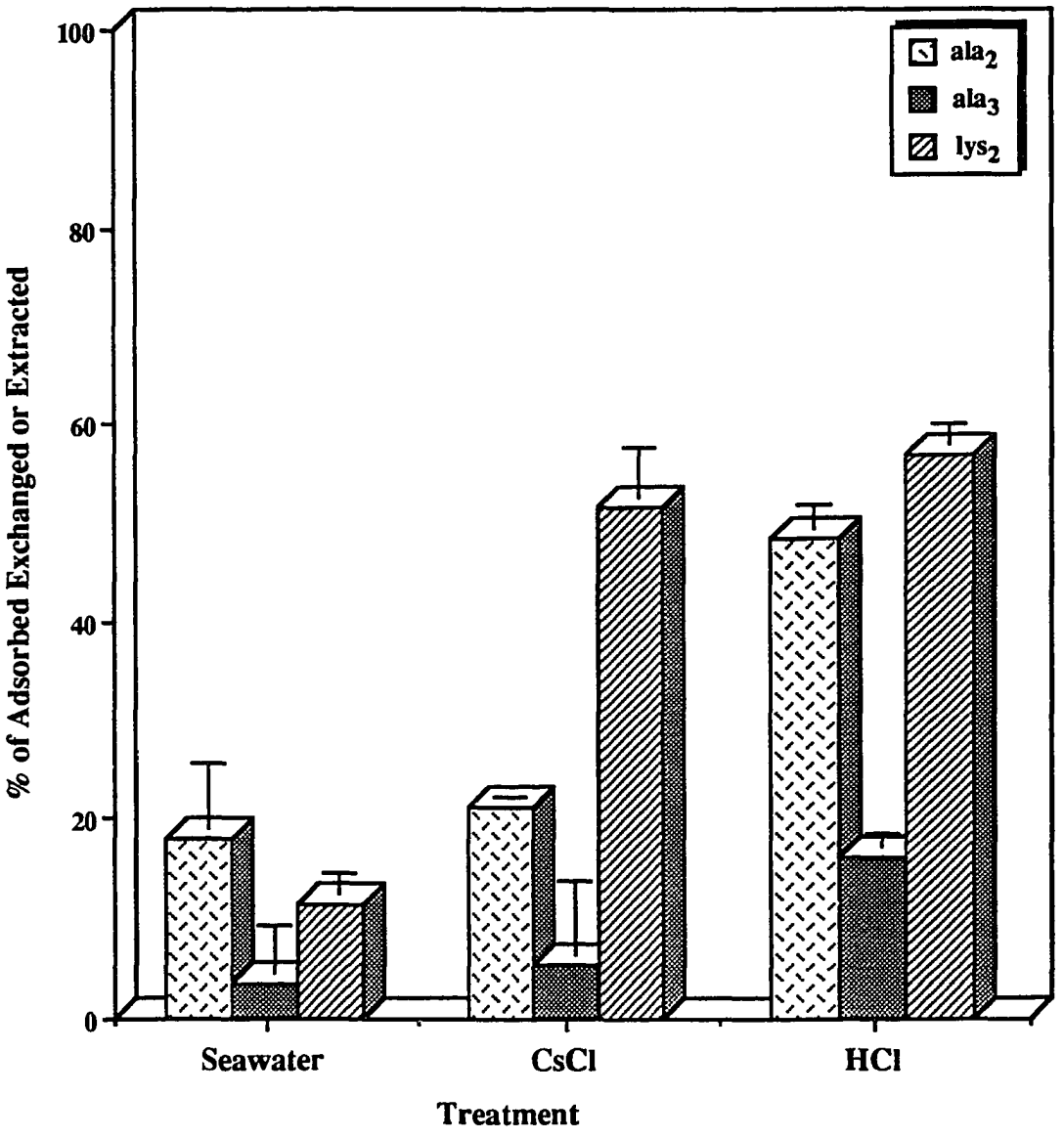


Figure 2.21. Exchange and Extraction of Adsorbed ^{3}H-Peptides (at 3.33 μM Initial Concentration) from Skan Bay Sediments.

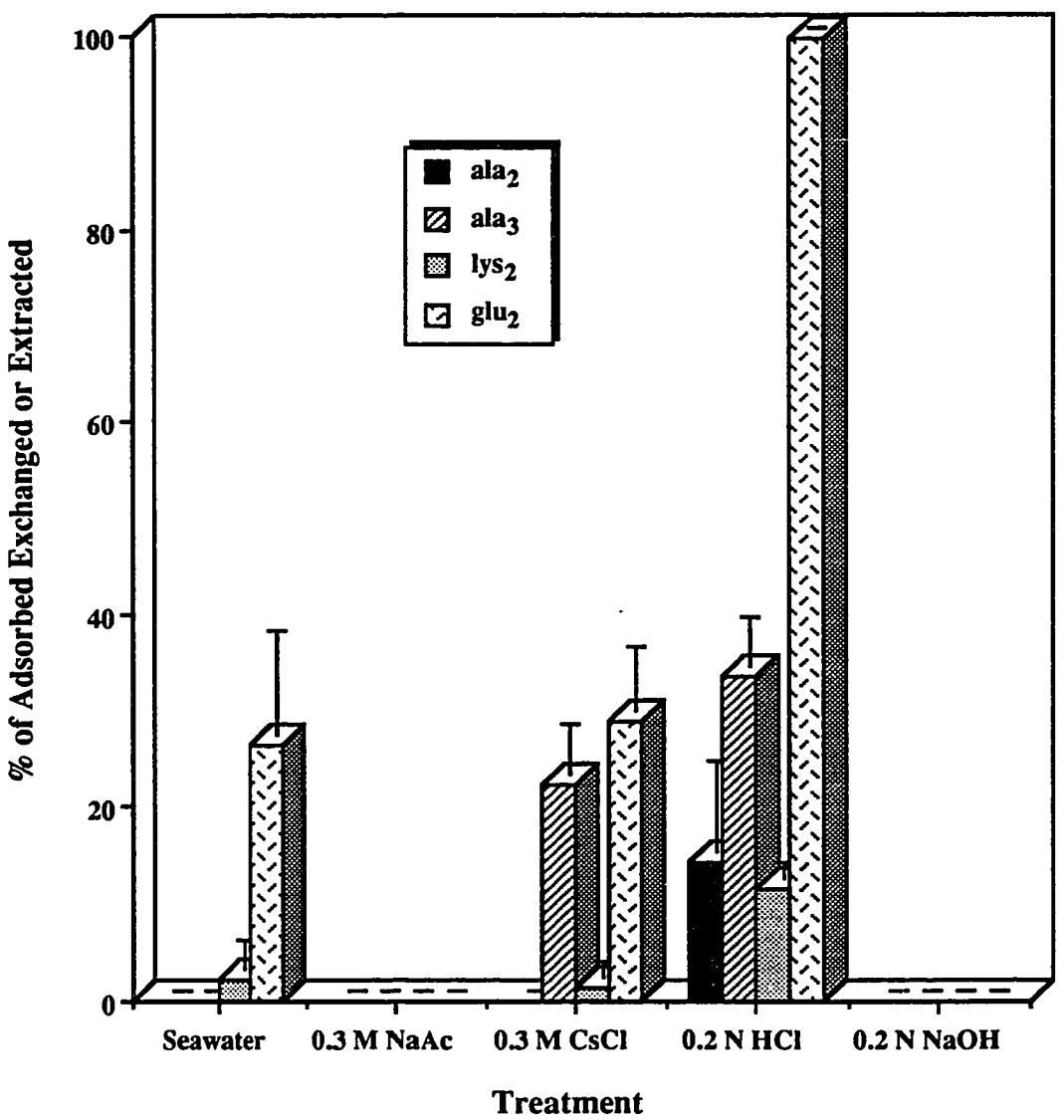


Figure 2.22. Exchange and Extraction of Adsorbed ^{3}H-peptides (at 3.33 μM Initial Concentration) from Autoclaved Skan Bay Sediments.

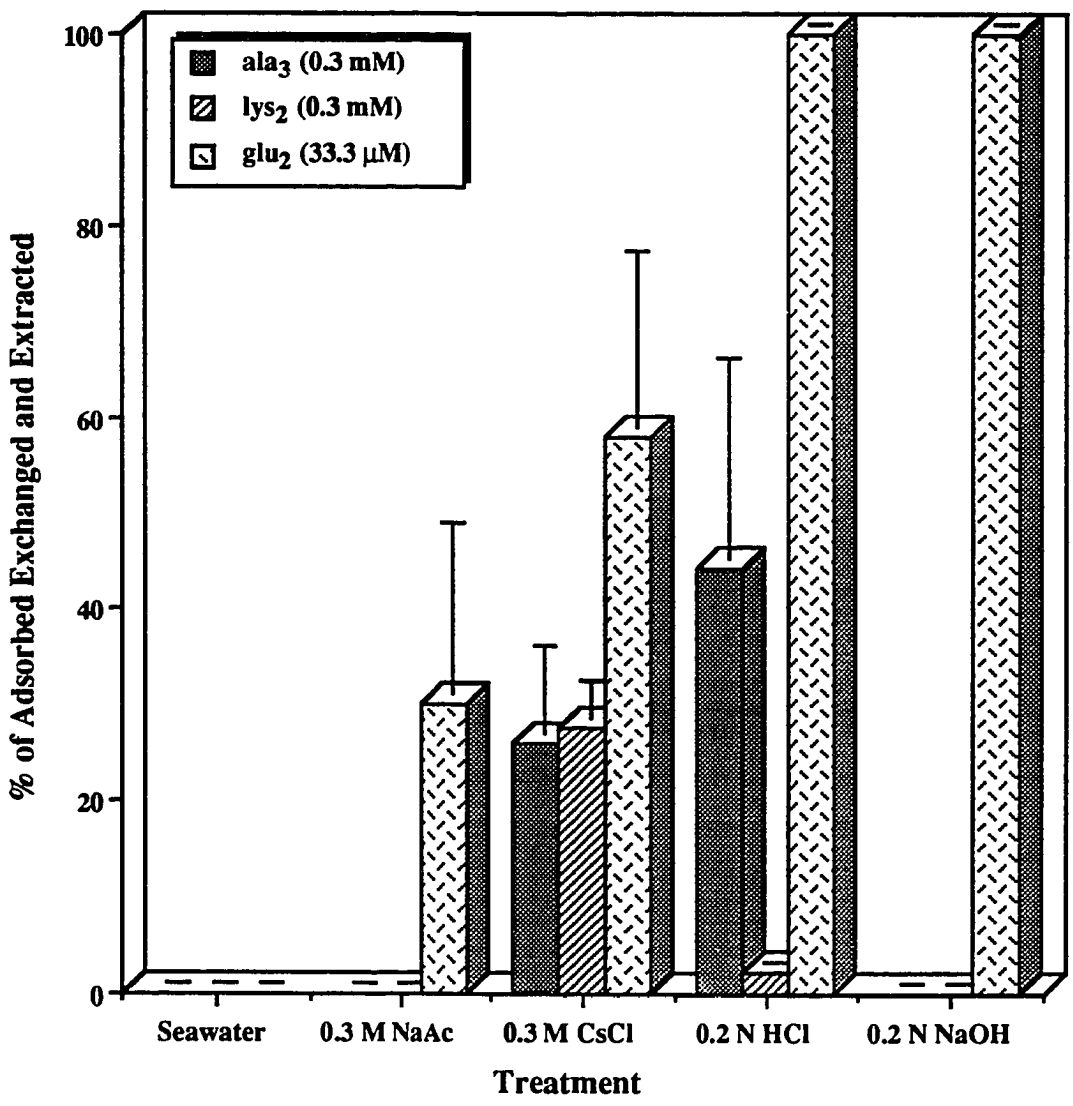


Figure 2.23. Exchange and Extraction of Adsorbed ^{3}H-Peptides from Autoclaved Skan Bay Sediments. Concentrations indicated in parentheses are the initial dissolved peptide concentration in pore water, before adsorption.

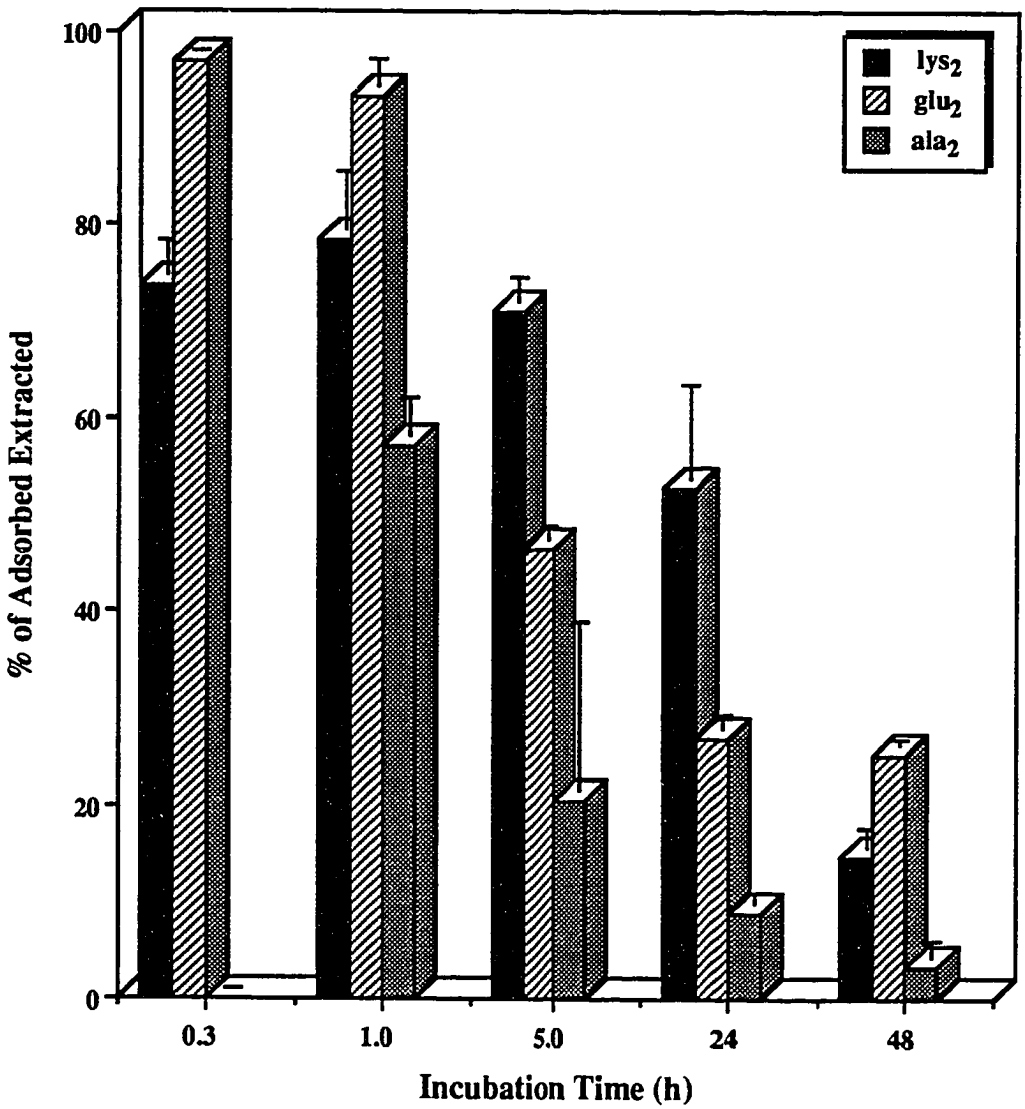


Figure 2.24. HCl Extraction of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) from Skan Bay Sediments.

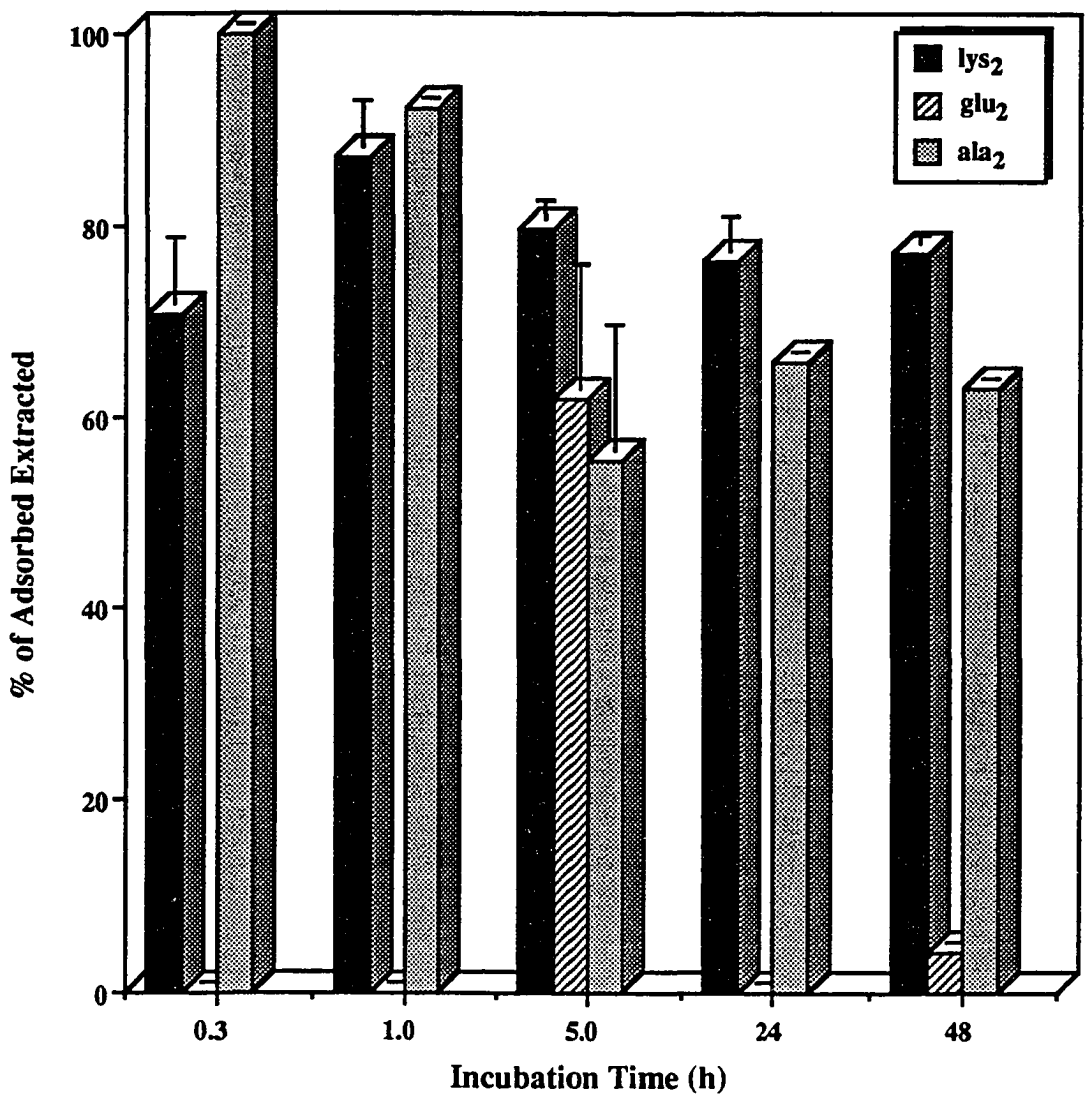


Figure 2.25. HCl Extraction of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) from Autoclaved Skan Bay Sediments.

Decomposition of the adsorbed ^{3}H-peptides Since adsorption of ^{3}H-ala_3 and ala_6 in autoclaved sediments was very low, only the decomposition of adsorbed ^{3}H-ala_2, glu_2 and lys_2 was evaluated. After adsorption of the peptides to the autoclaved sediment, there were still some dissolved ^{3}H-peptides remaining. This remaining dissolved ^{3}H-peptide is defined as the initial ^{3}H-peptide, when fresh sediment was added and mixed with autoclaved sediment. If the adsorbed ^{3}H-peptides were re-dissolved in pore water and hydrolyzed, the total amount of dissolved ^{3}H-activity should increase with time. The total recovery of ^{3}H-peptide, in the dissolved, adsorbed and hydrolyzed pools, did increase slightly (Figure 2.26), indicating a decrease in the amount of adsorbed ^{3}H-peptides.

Figures 2.27 and 2.28 show the hydrolysis and respiration of adsorbed ^{3}H-peptides. The decomposition of adsorbed peptides increased gradually over time. Before 24 hours, the hydrolysis was in the order: $ala_2 \geq lys_2 > glu_2$. After 48 hours, the order changed to: $glu_2 > ala_2 > lys_2$. The respiration of ^{3}H-glu_2 was greatest after 5 hours. Less than 30% of the adsorbed ^{3}H-ala_2 and lys_2 was hydrolyzed within 48 hours, but almost all of the adsorbed ^{3}H-glu_2 was decomposed after 48 hours.

Figure 2.29 shows the variation of different dissolved ^{3}H-labeled fractions in the mixed autoclaved and fresh sediment during the incubation. Compared with in fresh sediment (Figure 2.9), the pattern of variation was similar, except the dissolved ^{3}H-peptide loss from the pore water of the mixed sediment was slower than that from fresh sediment, and the increase in ^3H_2O in mixed sediment was less.

Effect of added ^{3}H-activity on data accuracy The hydrolysis and adsorption of ^{3}H-peptide were compared in experiments using different ^{3}H-activities in the added solutions to see if the added activity would affect the accuracy of the measurements (Table 2.1). Theoretically, a higher activity in solution should increase the measurement

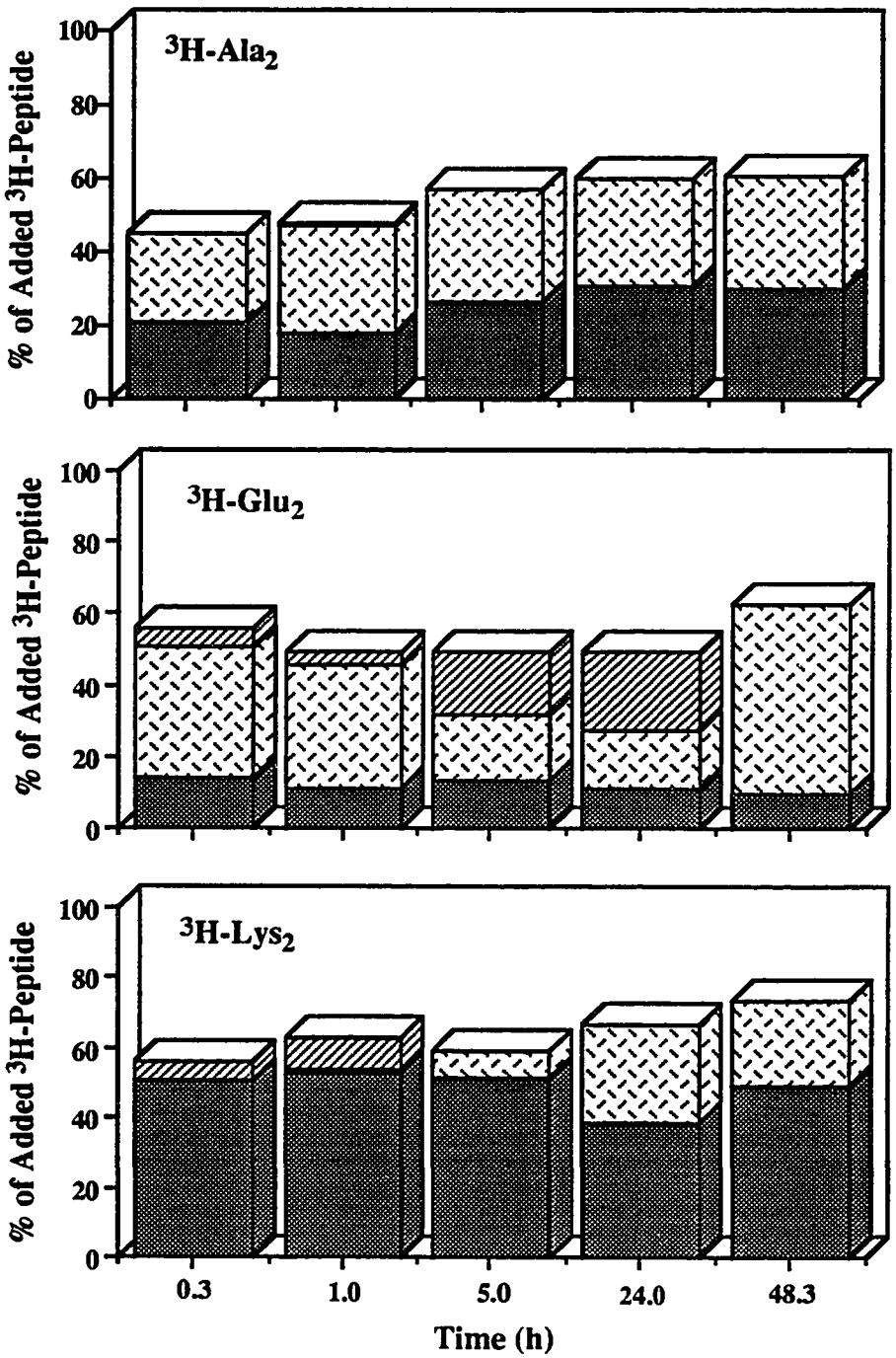


Figure 2.26. ^{3}H-Peptides (at 0.01 μM Initial Concentration) in Skan Bay Sediments (Autoclaved + Fresh).
▨ ^{3}H-peptide adsorbed ▢ ^{3}H-peptide hydrolyzed
■ ^{3}H-peptide remaining in porewater

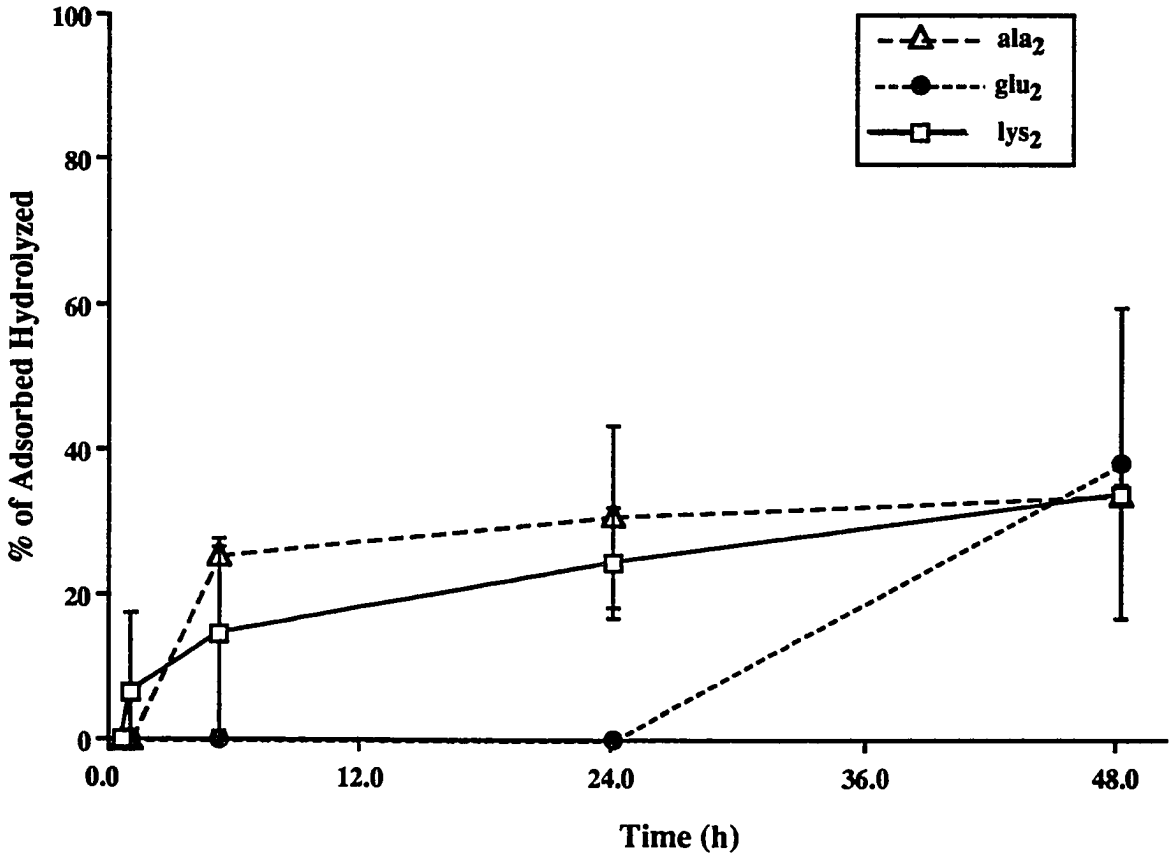


Figure 2.27. Hydrolysis of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Skan Bay Sediments.

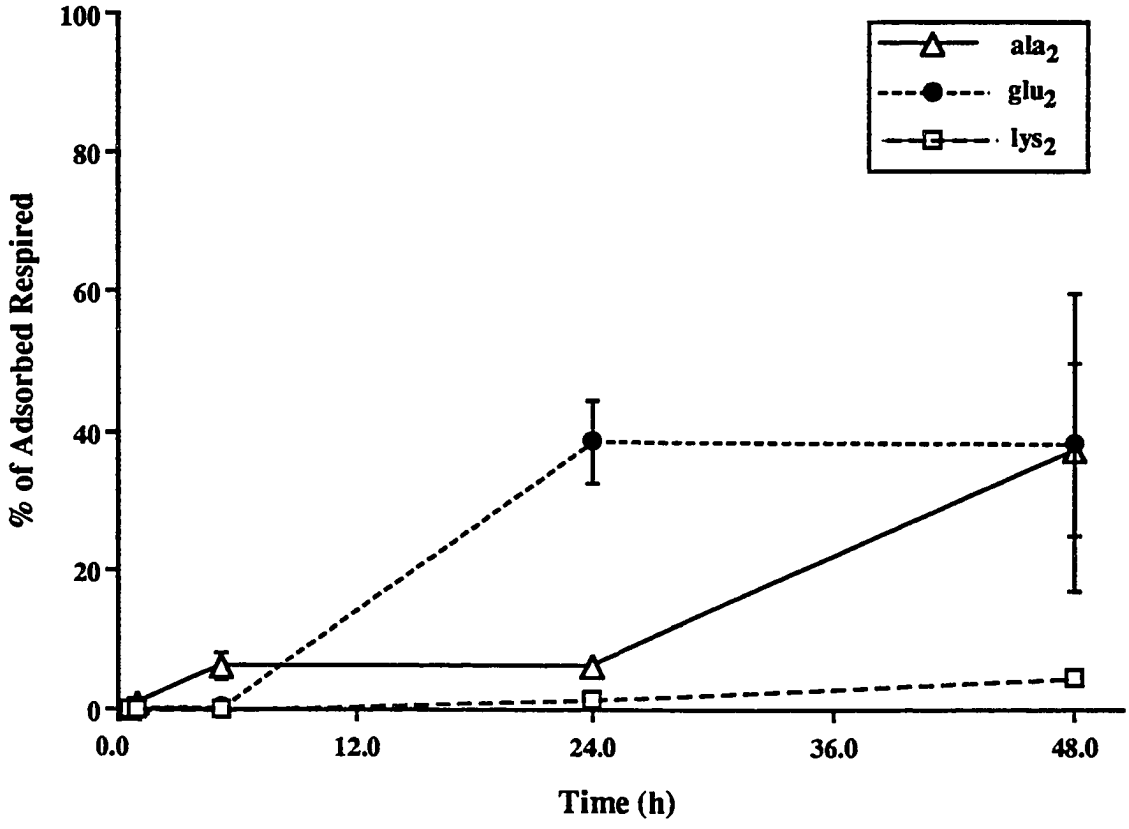


Figure 2.28. Respiration of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Skan Bay Sediments.

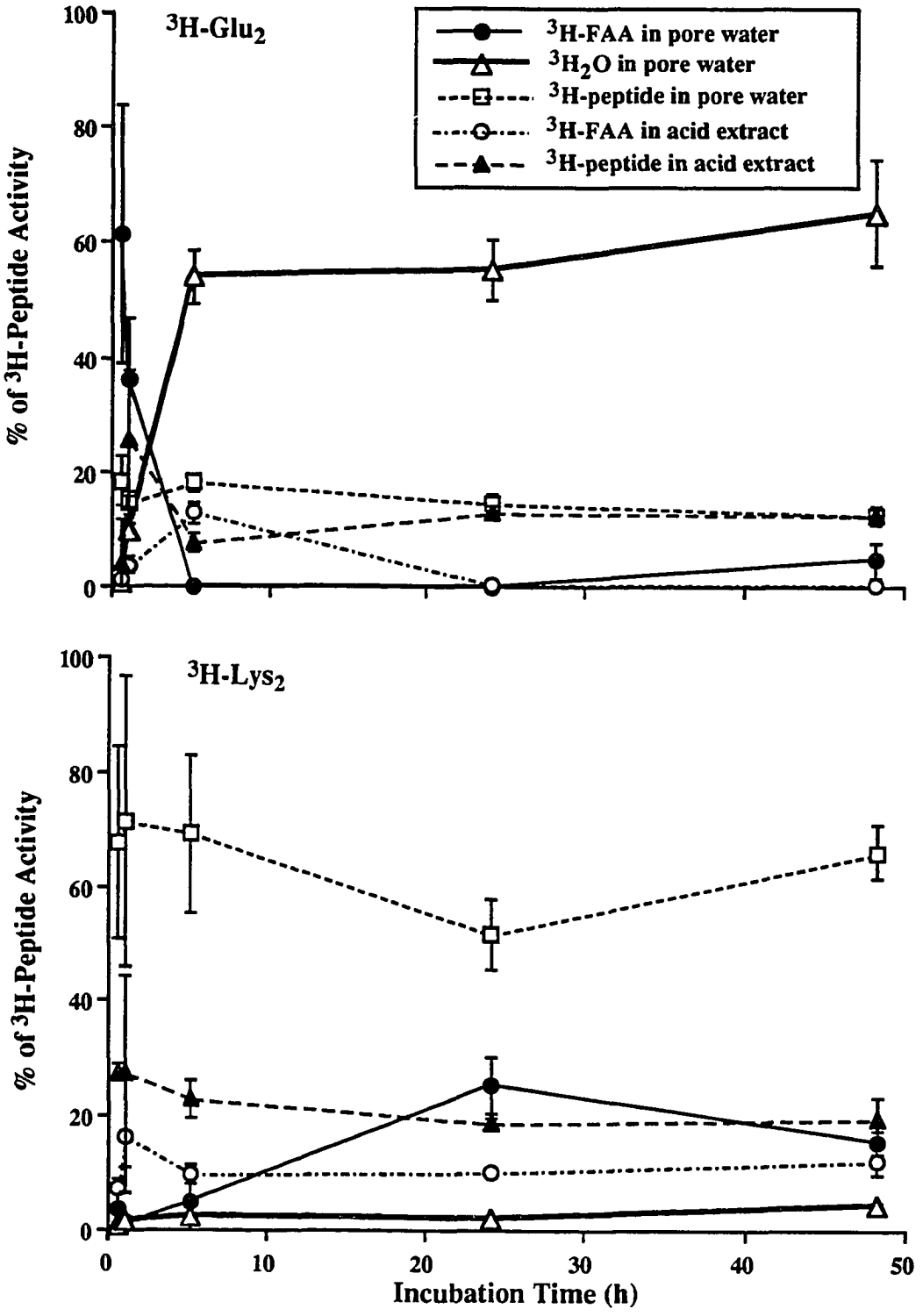


Figure 2.29. Decomposition of ^{3}H-Peptides (at 0.01 μM Initial Concentration) in Skan Bay Sediments (Autoclaved + Fresh).

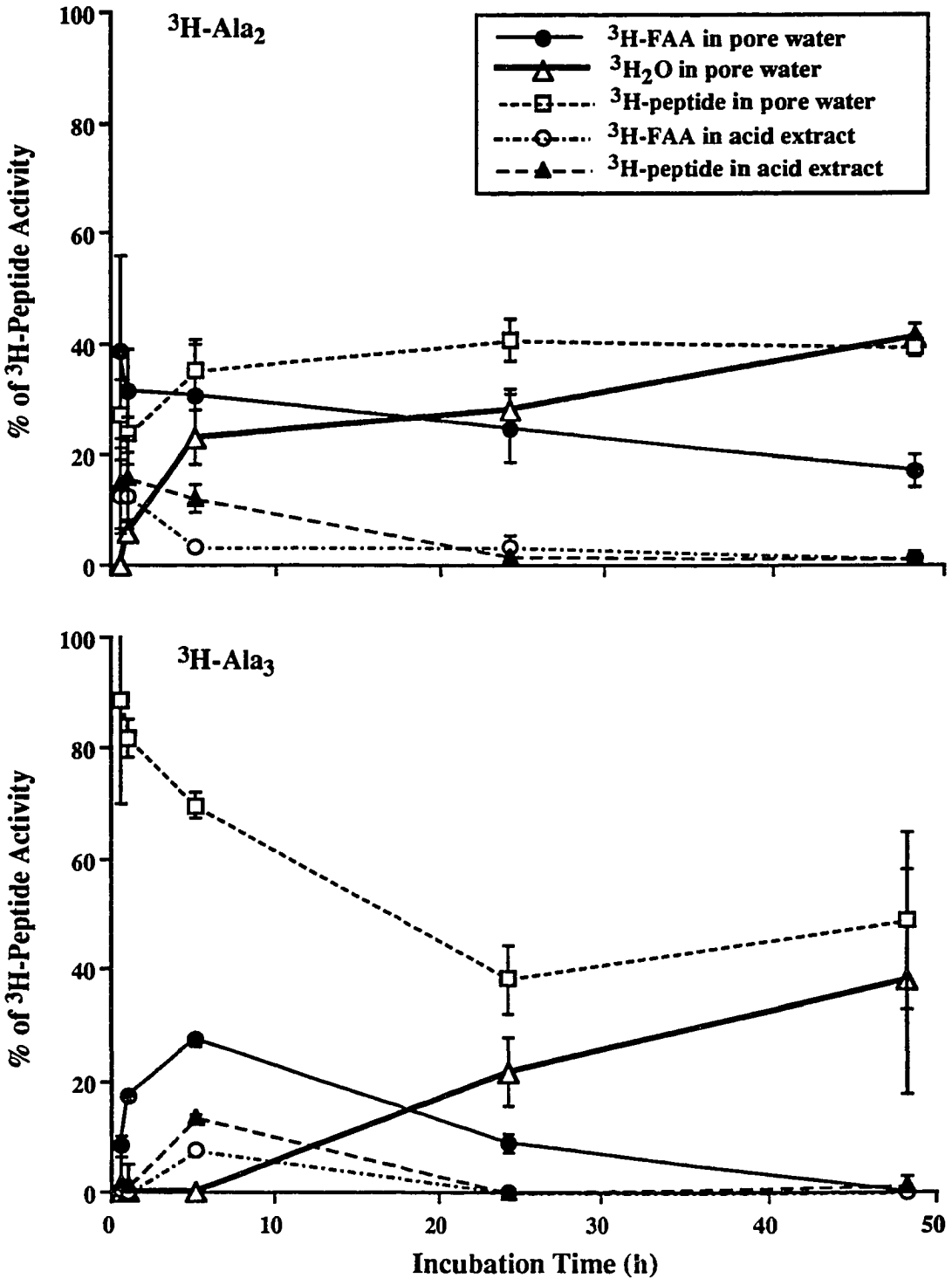


Figure 2.29. (continued)

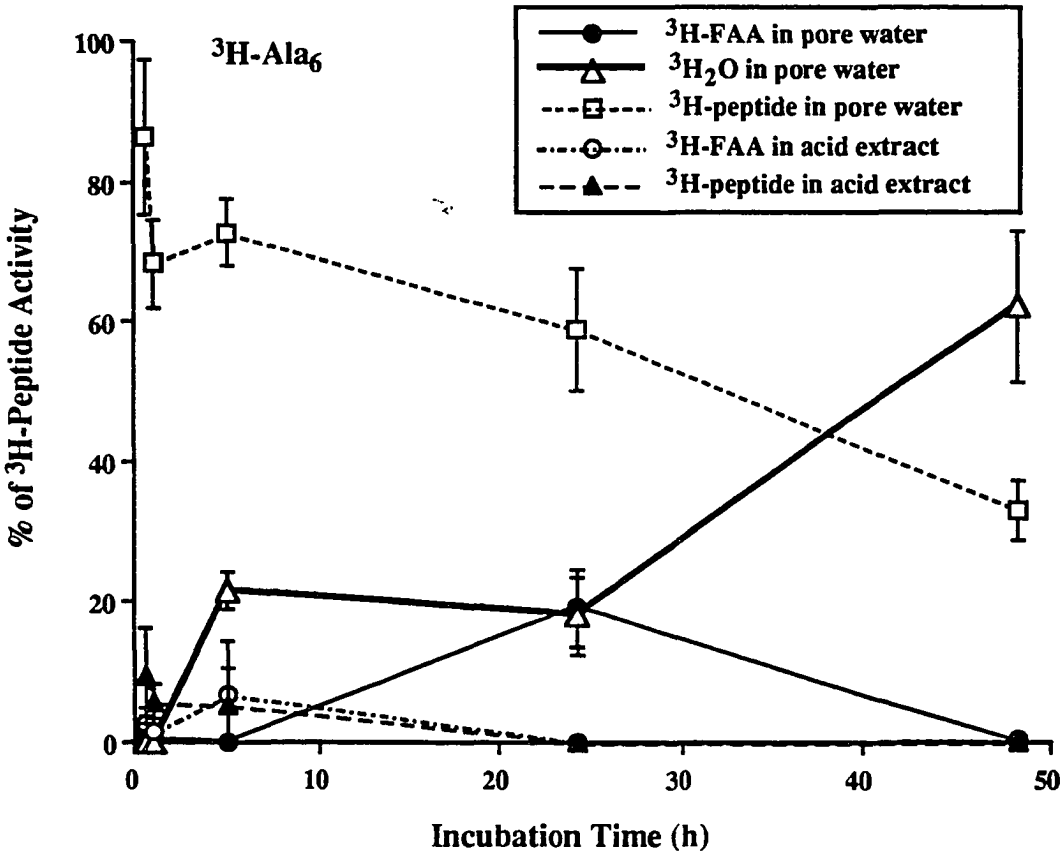


Figure 2.29. (continued)

Table 2.1. Effect of ^3H-Activity in Added Solutions

date[1]	^3H-activity in added solutions[2] ($x10^3$ dpm/mL)	% of hydrolysis[3]
April 20	di-glutamic acid (0.3)	79.9±10.1
October 15	di-glutamic acid (17.3)	60.8±7.8
June 2	di-lysine (1.0)	51.4±1.6
June 18	di-lysine (10.7)	35.7±1.7
October 15	di-lysine (8.3)	28.9±8.7
May 15	di-alanine (0.7)	65.6±3.9
June 17	di-alanine (7.7)	61.4±7.4
October 15	di-alanine (5.7)	53.3±4.3
May 14	tri-alanine (1.3)	49.2±1.2
June 17	tri-alanine (3.7)	37.2±4.0
September 2	tri-alanine (3.3)	69.4±17.4
October 15	tri-alanine (3.3)	28.4±1.4
June 2	hexa-alanine (0.7)	59.7±4.2
June 18	hexa-alanine (3.7)	100
October 15	hexa-alanine (3.3)	32.5±3.4

[1] The experiment in October used fresh sediments. Measurements at other times used stored sediments (see text).
[2] The concentration of peptide was 0.03 μM. The incubation time was 18 min.
[3] Data given as mean±S.D. (n=3).

sensitivity. However, within the ^{3}H-activity range in the added solutions, the variability of results was not correlated with the amount of added activity. The comparison of the variability of adsorption and hydrolysis vs. sample, core, and added ^{3}H-activity (APPENDIX III, Table A.2) also indicated that the variability is probably due to sediment heterogeneity rather than experimental error.

Clay mineral analysis Clay was found to be 5 - 6% of the dry sediment weight. The composition of clay minerals shown by x-ray diffraction was: no expandable minerals/smectite, trace amount of kaolinite, 22% illite, and 78% chlorite, this indicates that Skan Bay sediment was derived from physical weathering processes.

Discussion

Hydrolysis and respiration in pore water Rapid enzymatic hydrolysis of ^{3}H-glu_2 and ^{3}H-ala_2 occurred in 0.2-μm filtered pore water (Figures 2.3, 2.4 and 2.5), apparently due to dissolved, extracelluar enzymes. Although bacteria < 0.2 μM diameter exist in seawater (e.g., Robertson and Button 1989), they have not been reported in sediment. The data also indicate the apparent cessation of hydrolysis after 1 hour. One possible explanation is that the hydrolytic activity of enzymes is controlled by the concentration of glu_2 and ala_2 in pore water. However, it does not seem likely that the hydrolysis rate would decrease so much and so rapidly due to this cause when about half of the added peptides still remained. The possibility that other radiolabeled substances (i.e., D-amino acid peptides) were added in addition to L-ala_2 and L-glu_2 peptides was also investigated. D-peptides are hydrolyzed more slowly than L-peptides by enzymes. However, 93% of added ^{3}H-glu_2 and 100% of added ^{3}H-ala_2 were recovered in acid hydrolyzed solutions as L-glutamic acid and L-alanine (APPENDIX III, Table A.7, analytical techniques from Nimura *et al.* 1981 and Robertson *et al.* 1987). Other authors

have proposed that amino acids or acetate form "complexes" in pore water which are unavailable to microorganisms (Christensen and Blackburn 1980; Ansbaek and Blackburn 1980). However, no evidence for such complexes has been found in Skan Bay pore water (McDaniel 1989; Shaw and McIntosh 1990).

For ^{3}H-glu$_2$, the hydrolysis rate increased with concentration (nM) (Figure 2.6a), without a "critical concentration". For this pattern, interpretation according to a diffusion kinetic model (Wright and Hobbie 1965) was suggested by Wright and Burnison (1979). By plotting concentration (V in nM) vs. hydrolysis rate (R_H in nmol L^{-1} d^{-1}), two straight lines with different slopes were obtained (Figure 2.6a). The equations for these lines are:

$$R_H = 3.72x10^3 + 2.89\ V \quad (r^2 = 1.000)\ (15\ nM\ to\ 4.5\ mM)$$

$$R_H = 9.09x10^6 + 0.83\ V \quad (r^2 = 1.000)\ (4.5\ mM\ to\ 45\ mM)$$

The slope of the straight line is the diffusion constant k_d of ^{3}H-peptides in units of day^{-1} (d^{-1}). Two distinct k_d values were measured: 2.89 d^{-1} (0.12 h^{-1}) between 15 nM and 4.5 mM, and 0.83 d^{-1} (0.03 h^{-1}) between 4.5 mM and 45 mM. There probably are a variety of enzymes in pore water. Thus, the apparent k_d can change with concentration (Althel 1979).

For ^{3}H-ala$_2$, the hydrolysis rate reaches saturation at a concentration of 500 µM. A good linear correlation can be obtained with a Hanes-Woolf plot (Wright and Burnison 1979), plotting the ratio of concentration to hydrolysis rate vs. concentration (V/R_H vs V) (Figure 2.6b). The line fitted the equation derived from the Lineweaver-Burk transform of the Michaelis-Menten equation (Metzler 1977):

$$\frac{V}{R_H} = 0.58 + 1.69x10^{-6}\ V \quad (r^2 = 0.959)$$

For this equation, 1/intercept is the hydrolysis rate constant, 1.67 d^{-1} (0.07 h^{-1}); 1/slope is the maximum hydrolysis rate of ^{3}H-ala$_2$ (R_{Hmax}), 5.92x10^5 nmol L^{-1} d^{-1}.

The hydrolysis rate at the saturation concentration was $6.0x10^5$ nmol L^{-1} d^{-1}, and the concentration corresponding to the measured half maximum hydrolysis rate ($3.0x10^5$ nmol L^{-1} d^{-1}) was $0.3x10^6$ nM (the dashed line in Figure 2.6b).

The peptide molecule has to be first bound to an enzyme in order to be hydrolyzed. For particular enzymes, binding may occur at either the N- or C-terminal of the peptide. If binding can occur only at the C-terminal for the enzymes present in sediments, the probability of detectable hydrolysis for all the ^{3}H-peptides should be equal, because the peptides were C-terminal ^{3}H-labeled. If the binding can occur only at the N-terminal of the peptides, the probability of hydrolysis of ^{3}H-ala$_3$ and ^{3}H-ala$_6$ to ^{3}H-alanine is only one half and one fifth that of ^{3}H-ala$_2$. However, the measured hydrolysis rates of ^{3}H-ala$_3$ and ^{3}H-ala$_6$ were less than half and one fifth that of ^{3}H-ala$_2$ during a 24 hour-incubation. Thus, no matter what the mechanism, hydrolysis of ^{3}H-ala$_3$ and ^{3}H-ala$_6$ was less than that of ^{3}H-ala$_2$. The enzymatic hydrolysis rate of peptides in solution generally decreases with increasing molecular size, because the rate of formation of productive enzyme-substrate complexes is less (Metzler 1977). However, the rate differences among the peptides are again too large to be explained solely by this effect.

Regardless of how the peptide is bound to the enzyme, the probability of detectable hydrolysis is equal for di-peptides. Thus, the differences in the hydrolysis rates among di-peptides must be due to other reasons. For example, bacterial cells may produce enzymes selective for certain amino acids. According to this idea, sediment bacteria may require more glutamic acid and alanine than other amino acids, thus resulting in high hydrolysis rates of ^{3}H-glu$_2$ and ^{3}H-ala$_2$. Glutamic acid and alanine are the predominant dissolved free amino acids in the pore water of Skan Bay and most other anoxic marine sediments (Henrichs and Farrington 1987; Burdige and Martens

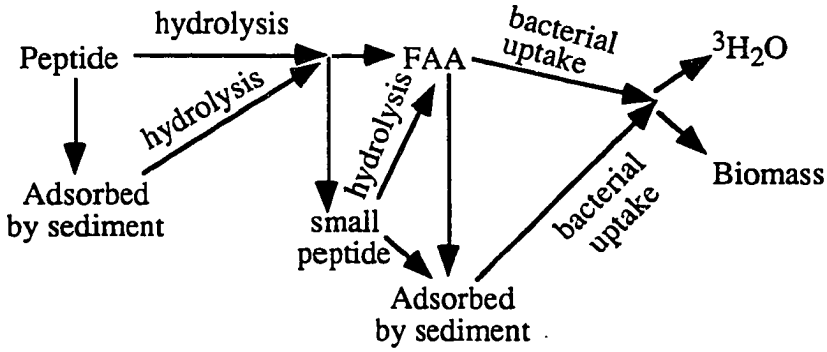
1988; McDaniel 1989; Burdige 1991; Sugai and Henrichs, in preparation). The production of dissolved free amino acids (DFAAs) is thought to occur through the hydrolysis of protein in the sediments, presumably by fermentative proteolytic bacteria (Reichardt 1986; Mayer 1989). If the higher pore water concentrations of glutamic acid and alanine result from the selective hydrolysis of proteinaceous material, then the rapid hydrolysis of ^{3}H-glu$_2$ and ala$_2$ in pore water would be expected.

Although there was rapid hydrolysis of ^{3}H-glu$_2$ and ala$_2$ in pore water, there was no production of ^3H_2O within 24 hours indicating that the hydrolysis products (FAA) were slowly respired. McDaniel (1989) also found that glycine, serine, alanine, glutamic acid and lysine were slowly respired in 5 μM-filtered Skan Bay pore water, with the average time for 50% removal being 140 hours.

The results of peptide decomposition measurements in Skan Bay pore water are quite difficult to interpret. It is likely that the rapid hydrolysis of ala$_2$ and glu$_2$ was effected by extracelluar enzymes in the pore water. However, the kinetics of enzyme hydrolysis cannot be entirely explained by simple models. The major oddity was that the rates of glu$_2$ and ala$_2$ hydrolysis decreased more than 100 times after the first 20 minutes, when only about half of the added peptide was consumed. Although there is no direct evidence, the only explanation that seems reasonable is that ala$_2$ and glu$_2$ were somehow rendered unreactive via reaction with some pore water constituent. An additional possibility is that enzyme concentrations were very low and some small impurity in the peptide solution was bound irreversibly. However, this seems unlikely because enzyme saturation occurred only at high peptide concentrations. Also, the differences in hydrolysis rates among ala$_2$, ala$_3$ and ala$_6$ were too great to be explained unless the enzymes could hydrolyze only the di-peptides. Enzymes effective for the larger peptides might have been absent, or there may have been greater dilution of the

labeled substrates with analogous substances in pore water. Another explanation is that the smallest peptides were taken up by very small bacteria, before hydrolysis, but it then becomes difficult to explain why little or no respiration occurred.

Hydrolysis and respiration in sediment The decomposition pathway of polypeptides or proteinaceous materials involves hydrolysis to FAA, before metabolism to CO_2 and H_2O. In my experiments, ^3H-peptides were decomposed following this route because the ^3H_2O production occurred only after the accumulation of ^3H-FAA (Figure 2.30). The fate of peptides in sediment is illustrated below:



The amount of the hydrolysis product in the dissolved pool depends on the rates of peptide hydrolysis, as well as the rates of ^3H-FAA adsorption and respiration in sediments. If FAA respiration is very rapid compared to peptide hydrolysis, or FAA adsorption is very rapid, there will be no accumulation of FAA in pore water. Figure 2.30 shows the variation of ^3H-peptide, ^3H-FAA and ^3H_2O in solution during decomposition in sediment. For ^3H-lys_2, glu_2 and ala_2, the total amount of ^3H_2O produced was equal to the amount of ^3H-FAA that once existed in the dissolved pool. The accumulation of ^3H-lysine over time corresponded very well with ^3H_2O production while the accumulation of ^3H-glutamic acid and alanine occurred only at 15 minutes, i.e., ^3H-alanine and glutamic acid were removed from the dissolved pool faster than ^3H_2O was produced. This same pattern has been seen for $^{14}CO_2$ production from ^{14}C-

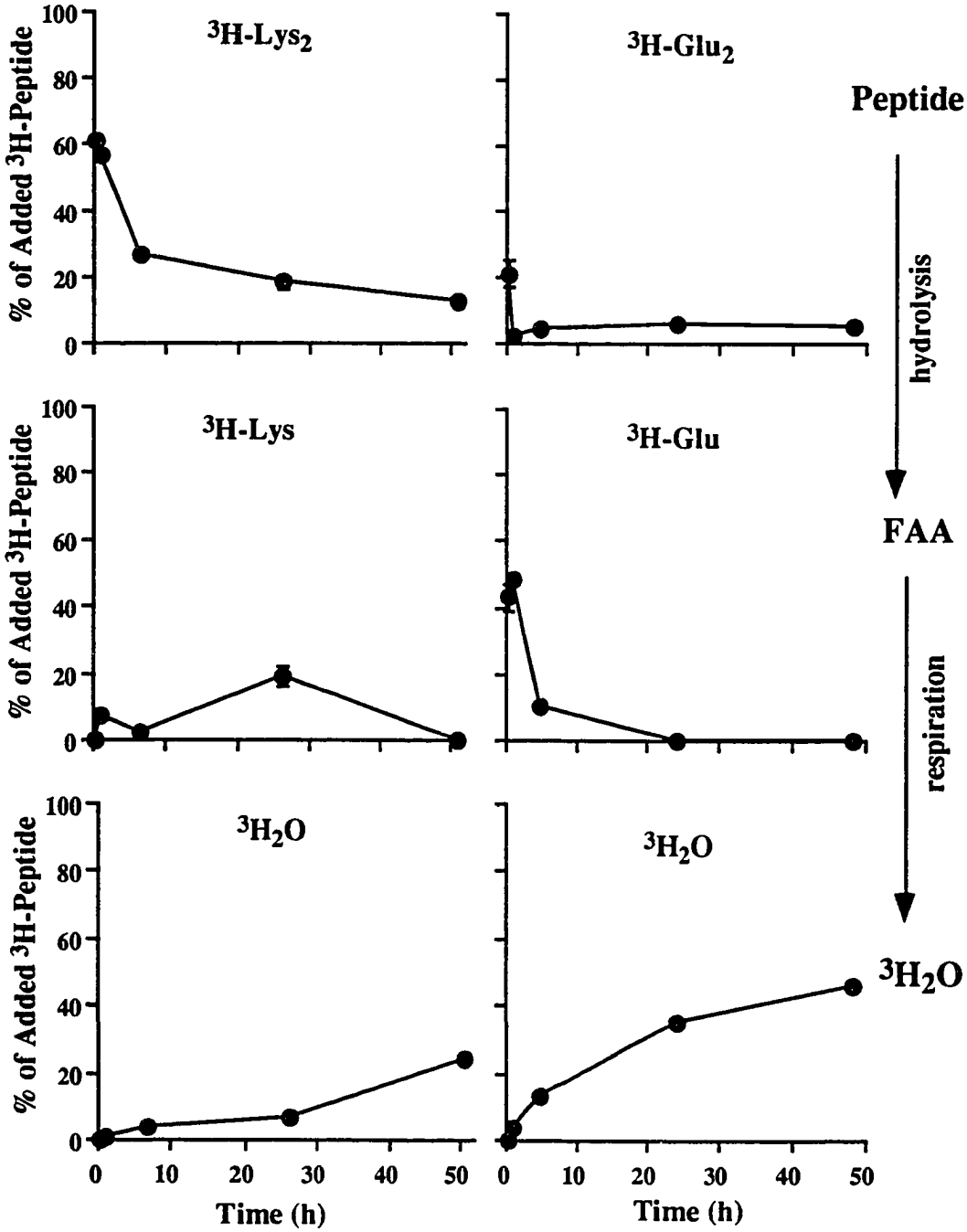


Figure 2.30. Decomposition Pathway of ^{3}H-Peptides in Skan Bay Sediments.

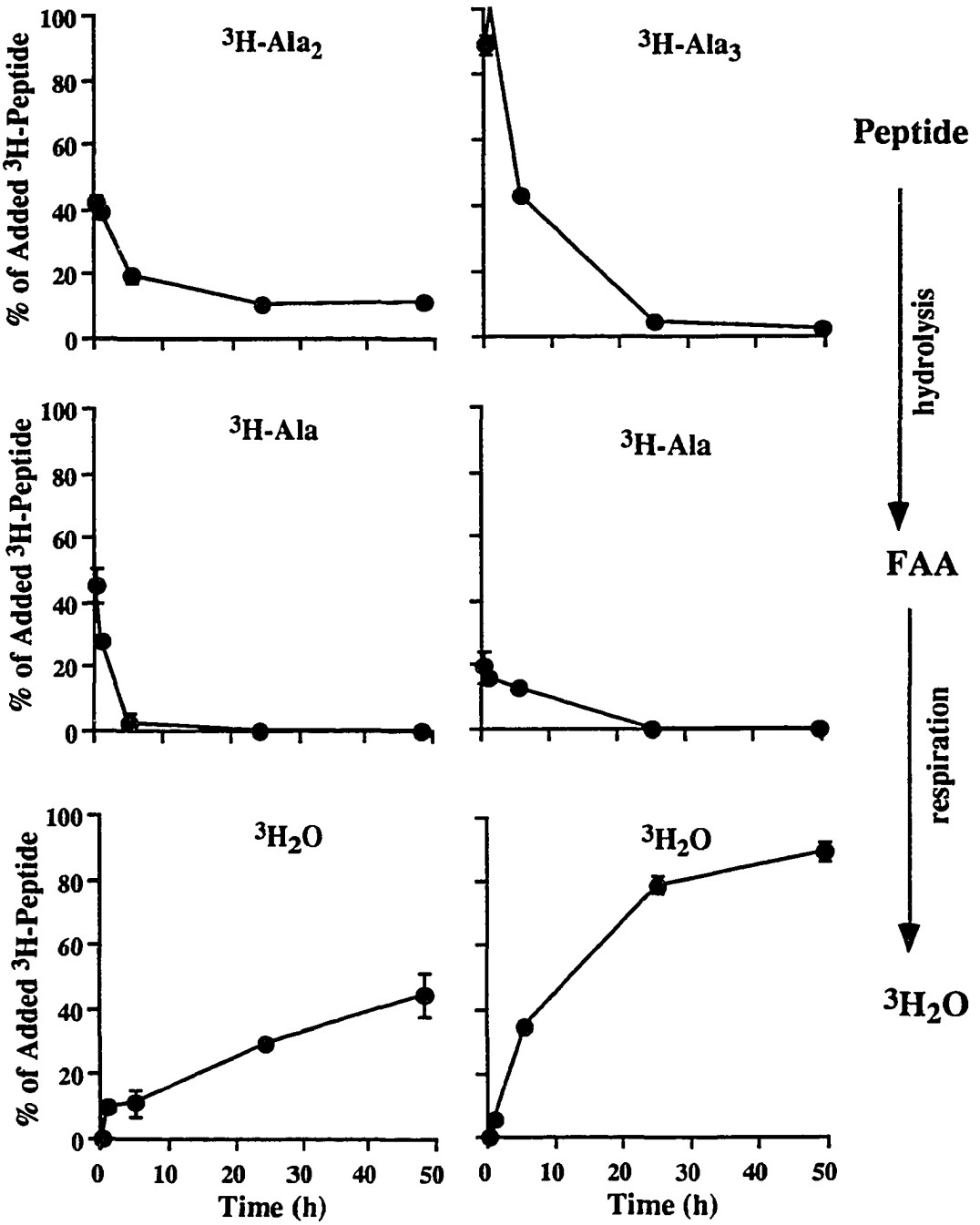


Figure 2.30. (continued)

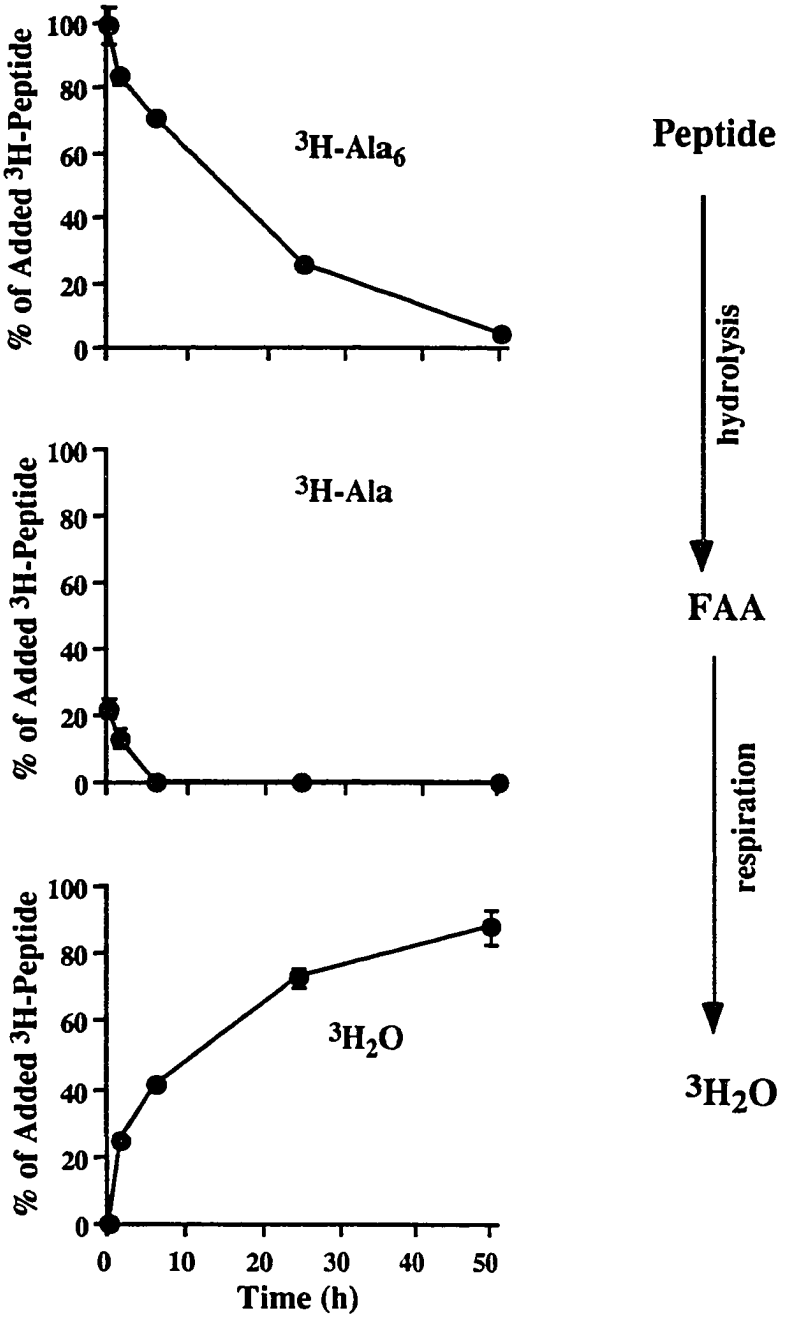


Figure 2.30. (continued)

labeled free amino acids (Sugai and Henrichs, in preparation). For ^{3}H-ala$_3$ and ala$_6$, the total amount of ^3H_2O produced was four times greater than the amount of hydrolyzed FAA in the dissolved pool and FAAs were again removed from the dissolved pool faster than ^3H_2O was produced. Respiration still followed hydrolysis, because no ^3H_2O appeared until ^{3}H-alanine was released to the dissolved pool. However, the relatively small ^{3}H-FAA production suggested that more of the ^{3}H-alanine hydrolyzed from ^{3}H-ala$_3$ and ala$_6$ was taken up into the intracellular pools of bacteria than was FAA from ^{3}H-lys$_2$, glu$_2$ and ala$_2$.

Rates of hydrolysis and respiration can be calculated by using models. Two methods have been used to measure the rates of labeled substrate turnover in sediments. The first involves addition of several concentrations of labeled substrate greater than ambient concentrations. Michaelis-Menten kinetics are employed to calculate the rate parameters at the *in situ* concentration of substrate (e.g., Williams 1973; Fenchel and Jørgensen 1977; Christensen and Blackburn 1980). The second method uses a tracer model, and labeled substrate is added at a concentration much lower than that in sediments (e.g., Hanson and Gardner 1978; Sugai and Henrichs 1992). Both of these methods were applied to measure the turnover rates of ^{3}H-peptides in sediments.

The kinetic model (Wright and Burnison 1979) previously used for the pore water was also applied to sediment. The rate constant of hydrolysis (k_d) is equal to the slope/(ϕ * 24) in units of hours^{-1} (Table 2.2). Among ^{3}H-peptides, the order of k_d was: glu$_2$ > ala$_2$ > ala$_3$ > ala$_6$ > lys$_2$. ^{3}H-glu$_2$ was hydrolyzed 3 to 4 times faster than the other ^{3}H-peptides in sediments. For the first 15 to 20 minutes, the hydrolysis rate constants for peptides were much greater in sediment than in pore water. This indicated that most of the hydrolytic enzyme activity in this sediment is associated with sediment particles or bacterial cells. In sediments, as in pore water, there is a marked decrease in

Table 2.2. Rate Parameters of Peptide Decomposition in Skan Bay Sediments

^{3}H-peptide	direct measurement[1]	diffusion model[2]	tracer model[3]							
	k_{dm} (h^{-1})	k_d (h^{-1})	k_1	k_2	k_3	R	k_p	k_a	k_{PAE}	S
			(h^{-1})							
di-alanine	1.843±0.143	0.563	0.30	0.01	0.02	0.40	0.30	0.03	0.01	5.95
tri-alanine	0.947±0.047	0.473	0.25	0.02	0.02	0.20	0.20	0.01	0.02	6.54
hexa-alanine	1.083±0.113	0.478	0.30	0.02	0.03	0.20	0.10	0.005	0.02	5.91
di-lysine	0.963±0.290	0.327	0.09	0.07	0.06	0.75	0.07	0.60	0.01	5.29
di-glutamic acid	2.027±0.260	1.420	0.50	0.03	0.20	0.60	1.0	0.06	0.04	6.99

[1] k_{dm} is the rate constant of ^{3}H-peptide hydrolysis calculated by [% of hydrolysis]/t. Data are given as mean±S.D. (n=3).
[2] k_d is the rate constant of ^{3}H-peptide hydrolysis calculated using the Wright and Burnison (1979) model.
[3] k_1, k_2, k_3, R, k_p, k_a and k_{PAE} are parameters defined in the text. S is the error of the model fit to the data, the square root of the sum of squared residuals divided by the number of data points, in units of % of added radioactivity.

hydrolysis rate with time. However, in sediments, there is a corresponding decrease in dissolved peptide concentration.

The following tracer model was applied to the 48-hour experiments using low added peptide concentrations (Figure 2.9), in which rates of hydrolysis and adsorption of ^{3}H-peptides, as well as the rate of bacterial uptake, adsorption and mineralization of the hydrolysis products (FAAs), were estimated. The model parameters are defined as follows:

C_{FAA} = % of added radioactivity as ^{3}H-FAA in pore water

C_P = % of added radioactivity as ^{3}H-peptide in pore water

C_{AE} = % of added radioactivity recovered as ^{3}H-FAA in acid extract

C_{PAE} = % of added radioactivity recovered as ^{3}H-peptide in the acid extract

^3H_2O = % of added radioactivity recovered as ^3H_2O

t = time since the ^{3}H-peptide was added

k_1 = rate constant for bacterial uptake of ^{3}H-FAA from pore water

k_2 = rate constant for bacterial uptake of ^{3}H-FAA from acid-extractable pool

k_3 = rate constant for ^{3}H-FAA adsorption plus bacterial uptake of ^{3}H-FAA from pore water into the acid-extractable pool

k_P = rate constant for ^{3}H-peptide hydrolysis in pore water

k_a = rate constant for peptide adsorption

k_{PAE} = rate constant for adsorbed ^{3}H-peptide hydrolysis

R = (activity in insoluble biomass) (^3H_2O + biomass activity)$^{-1}$

$$\frac{d C_{FAA}}{d t} = -k_1 C_{FAA} - k_3 C_{FAA} - \left(\frac{d C_P}{d t} + \frac{d C_{PAE}}{d t}\right) \qquad \text{----[7]}$$

$$\frac{d C_{AE}}{d t} = k_3 C_{FAA} - k_2 C_{AE} \qquad \text{----[8]}$$

$$\frac{d\,{}^3H_2O}{dt} = (1 - R)(k_1 C_{FAA} + k_2 C_{AE}) \qquad \text{----[9]}$$

$$\frac{dC_P}{dt} = -k_P C_P - k_a C_P \qquad \text{----[10]}$$

$$\frac{dC_{PAE}}{dt} = k_a C_P - k_{PAE} C_{PAE} \qquad \text{----[11]}$$

Combining [10] and [11] gives:

$$\frac{dC_P}{dt} + \frac{dC_{PAE}}{dt} = -k_P C_P - k_{PAE} C_{PAE} \qquad \text{----[12]}$$

The following are the solutions for Eqs. [7] to [12]:

$$C_P = 100\, e^{-(k_P + k_a)t} \qquad \text{----[13]}$$

$$C_{PAE} = B_2 e^{-k_{PAE} t} + \frac{k_a}{k_{PAE} - (k_a + k_P)} C_P \qquad \text{----[14]}$$

$$C_{FAA} = B_3 e^{-(k_1 + k_3)t} + \frac{B_2}{(k_1 + k_3) - k_{PAE}} e^{-k_{PAE} t} + \frac{k_P + \dfrac{k_a}{k_{PAE} - (k_a + k_P)}}{(k_1 + k_3) - (k_a + k_P)} C_P \qquad \text{----[15]}$$

$$C_{AE} = B_4 e^{-k_2 t} + \frac{B_3 k_3}{k_2 - (k_1 + k_3)} e^{-k_{PAE} t} + \frac{B_2}{(k_2 - k_{PAE})\left[(k_1 + k_3) - k_{PAE}\right]} e^{-k_{PAE} t} + \frac{k_3 + \left[k_P + \dfrac{k_a}{k_{PAE} - (k_a + k_P)}\right]}{\left[k_2 - (k_a + k_P)\right]\left[(k_1 + k_3) - (k_a + k_P)\right]} C_P \qquad \text{----[16]}$$

$${}^3H_2O = (1\text{-}R)(k_1 C_{FAA} + k_2 C_{AE})t \qquad \text{----[17]}$$

in which B_2, B_3 and B_4 are constant terms, which are different for different peptides (APPENDIX III, Table A.8).

Values for k_1, k_2, k_3, R, k_P, k_a, and k_{PAE} above were estimated by fitting Eqs. [13] through [17] to the data simultaneously. The best-fit was defined as minimization of the error (S) of the model fit to the measured data (Table 2.2). S, in units of % of added radioactivity, was equal to the square root of the sum of squared residuals divided by the number of data points, in which residuals were the differences between the measured radioactivity and the radioactivity calculated by fitting. The best-fit lines are shown in Figure 2.9. In this model, it is assumed that: (1) all tritium activity is either ^{3}H-peptide, ^{3}H-FAA or ^3H_2O during the experiment; (2) ^{3}H-peptides are hydrolyzed into ^{3}H-FAA before they are respired to ^3H_2O, i.e., direct bacterial uptake of ^{3}H-peptides is negligible (Figure 2.31). Also, ^3H_2O is produced only from dissolved free, intracellular or adsorbed ^{3}H-FAA, not via other radiolabeled substances in pore water or the acid-extractable fraction; and (3) ^{3}H-FAAs produced by ^{3}H-peptide hydrolysis are respired or adsorbed at the same rate as the ^{3}H-FAA in the added solution.

The hydrolysis rate constants (k_P) of ^{3}H-peptides calculated using the tracer model were in the order: glu_2 > ala_2 > ala_3 > ala_6 > lys_2 (Table 2.2). Compared with k_P, the hydrolysis rate constant of adsorbed peptides (k_{PAE}) was 10 to 30 times smaller. For alanyl peptides and glu_2, k_P is more than 10 times greater than the rate constant of peptide adsorption (k_a), and k_a is similar to k_{PAE}. However, for lys_2, k_a is about 10 and 60 times greater than k_P and k_{PAE}, respectively. Among the five peptides, k_{PAE} of glu_2 is relatively greater than that of other peptides, as was k_P. This may be one reason why glutamic acid is the predominant FAA in most Skan Bay pore water (Sugai and Henrichs, in preparation).

Since the amino acid released by hydrolysis of all alanyl peptides is alanine, the values of k_1, k_2, k_3 and R calculated by the model should be similar for ^{3}H-ala$_2$, ala$_3$ and ala$_6$, as observed (Table 2.2). Among the amino acids, k_1 varies: glutamic acid (0.5) > alanine (0.25 - 0.30) >> lysine (0.09), which is similar to previous results in Skan Bay pore water (McDaniel 1989). Also, R varies in the order lysine (0.75) > glutamic acid (0.60) > alanine (0.20 - 0.40); that is, lysine and glutamic acid were more efficiently incorporated into biomass than alanine.

The hydrolysis rate constant was also simply estimated by direct measurement (k_{dm}) of the % of peptide hydrolyzed over time (18 minutes) (Table 2.2). Different methods of calculating hydrolysis rates (k_{dm}, k_d, and k_P) all gave rate constants which decreased in the order glu$_2$ > ala$_2$ > ala$_3$ ≥ ala$_6$ > lys$_2$. However, for all ^{3}H-peptides except glu$_2$, the values of k_{dm} were 2 to 3 times greater than those of k_d, and the values of k_d were 2 to 5 times greater than those of k_P. Both k_d and k_{dm} are calculated only from results obtained for short incubations, while k_P is dependent on peptide concentrations measured over several hours; k_P thus tends to underestimate rapid initial hydrolysis rates. Also, the diffusion model and direct measurement might have oversimplified the peptide removal processes. Only the tracer model corrected for the effect of adsorption, so k_d and k_{dm}, which included effects of both hydrolysis and adsorption, should be greater than k_P. In addition, the calculation using the diffusion model was only based on the data at three concentrations, which led to a large uncertainty in the calculated k_d; the standard deviation was 20 to 50% of the average value (Table 2.2).

Theoretically, $C_P + C_{HFAA}$ = total activity of added peptide, where C_{HFAA} is the percentage of ^{3}H-amino acid hydrolyzed from ^{3}H-peptide, so

$$-\frac{dC_P}{dt} = \frac{dC_{HFAA}}{dt} \qquad \text{----[18]}$$

If hydrolysis is the rate-limiting step in the mineralization of ^3H-peptide, then

$$\frac{d\,^3H_2O}{dt} \geq \frac{d\,C_{HFAA}}{dt} \qquad \text{----[19]}$$

Combining Eqs. [18] and [19] gives:

$$-\frac{d\,C_P}{dt} \leq \frac{d\,^3H_2O}{dt} \qquad \text{----[20]}$$

i.e., the hydrolysis rate of peptide should be less than or equal to the respiration rate of amino acid.

The expression $(k_P+k_a)C_P^*$ from Eq. [10] can be used to calculate the hydrolysis rate of peptides. It is difficult to measure the concentrations of specific peptides in pore water (C_P^*) because there appears to be a complex mixture of peptides, or compounds with similar chromatographic properties, in pore water. The concentrations of all OPA-reactive substances which eluted at the same time as the studied peptides were measured. They varied from sample to sample: 0.29 - 0.61 μM for ala_2, 0.29 - 0.61 μM for ala_3, 0.05 - 2.45 μM for ala_6, 1.63 - 2.45 μM for glu_2 and 0.06 - 1.78 for lys_2. The calculated hydrolysis rates are showed in Table 2.3. The apparent hydrolysis rate of glutamyl peptide was greater that those of alanyl peptides and lysyl peptide. If the concentration of added peptide (C_{added}) instead of C_P^* is used, the calculated hydrolysis rates are much smaller (Table 2.3). It is reasonable to assume the peptide concentration is between C_{added} and C_P^*.

The respiration rates of FAA were calculated using the expression $24\phi(1-R)k_1C_{FAA}^*$ from Eq. [9] (Table 2.3). The FAA decomposition rates in Skan Bay surface sediments (0 to 3 cm) were previously reported as 7.2 nmol cm^{-3} d^{-1} for alanine and 0.72 to 4.0 nmol cm^{-3} d^{-1} for glutamic acid (Henrichs, unpublished data). For alanyl and glutamyl peptides, the range of respiration rates was similar to the range of hydrolysis rates. Thus, hydrolysis is apparently the rate-limiting step in the mineralization of these

Table 2.3. Decomposition Rates of Peptides in Skan Bay Sediments

peptide	hydrolysis rate[1] (nmol cm^{-3} d^{-1})		respiration rate[2] (nmol cm^{-3} d^{-1})
	$24\phi(k_P+k_a)C_P^*$	$24\phi(k_P+k_a)C_{added}$	
di-alanine	2.02 - 4.25	0.17	1.25 - 1.75
tri-alanine	1.29 - 2.71	0.11	1.39 - 1.94
hexa-alanine	0.11 - 5.43	0.05	1.67 - 2.32
di-lysine	0.85 - 25.19	0.34	0.03
di-glutamic acid	36.49 - 54.85	0.54	0.55 - 2.49

[1] Hydrolysis rate = $24\phi(k_P+k_a)C_P^*$, in which ϕ is 0.88, the sediment porosity (pore water volume/sediment volume); k_P and k_a are rate constants from Table 2.2; C_P^* is the peptide-like material concentration in pore water (see text). C_{added} (0.024 μM initially in pore water) is the concentration of added peptides.

[2] Respiration rate = $24\phi(1-R)k_1C_{FAA}^*$. k_1 and R are constants from Table 2.2; C_{FAA}^* is the concentration of FAA in pore water, 0.33 - 0.46 μM for alanine, 0.13 - 0.59 μM for glutamic acid and 0.06 μM for lysine (from Henrichs, unpublished data, and McDaniel 1989).

peptides. The hydrolysis rate of lysyl peptide was much greater than the respiration rate of the hydrolyzed product. This is probably because lysine is strongly adsorbed by sediments, and adsorbed lysine is mineralized much more slowly than dissolved lysine (Sugai and Henrichs 1992; Sugai and Henrichs, in preparation).

Some characteristics of enzymatic activity in sediments Hydrolytic enzyme activity in Skan Bay sediment could be inhibited only by autoclaving for two hours (Figure 2.12), and some autoclaved sediment retained low levels of enzyme activity even after this rigorous treatment (Figure 2.14). This occurred when larger volumes of sediment were treated, 500 mL instead of the usual 250 mL. However, even with the larger volume, 0.5 hour at about 120°C should have been sufficient to kill bacteria and denature most proteins (Korczynski 1981). Others have reported that freezing of the sediment does not significantly influence enzymatic activity; treatments with acetone, toluene, formaldehyde, glutaraldehyde or mercuric chloride only partly inhibited enzymatic hydrolysis; but boiling the sediment samples completely stopped hydrolysis (King 1986; Meyer-Reil 1990). Hydrolytic enzymes in Skan Bay sediment appear to have a structure which is very heat-stable.

The high enzymatic activity in stored sediment (two years old) is also difficult to explain; the rate of peptide hydrolysis was greater (except for ^{3}H-glu$_2$) than in fresh sediment (Figure 2.11). One possibility is that dead and decaying organisms contributed to the pool of extracellular enzymes (Mayer-Reil 1990). Another possibility is that, as readily metabolized peptides or protein decreased over time, more hydrolytic enzymes were produced by surviving organisms in an effort to break down the residual molecules.

Effects of autoclaving on adsorption of peptides in sediment The hydrolysis of ^{3}H-peptides in untreated sediments (especially in stored sediments) was so

rapid that a substantial amount of the added peptides was hydrolyzed to ^{3}H-FAA within 18 minutes (APPENDIX III, Table A.3). Thus, the adsorption process would be influenced by hydrolysis, resulting in differences in peptide adsorption by autoclaved and untreated sediments. However, for ^{3}H-lys$_2$, which was hydrolyzed relatively slowly compared to the other ^{3}H-peptides, the hydrolysis in untreated sediment was not much greater than that in autoclaved sediments for short incubations (Figures 2.18 and 2.19). Thus, adsorption of ^{3}H-lys$_2$ in autoclaved and untreated sediments was not significantly different (APPENDIX III, Table A.6), indicating that autoclaving did not substantially alter the chemical properties of the sediments important to lys$_2$ adsorption.

Autoclaving released some substances which could compete with peptides for binding sites. For example, autoclaving causes a large increase in pore water amino acid concentrations (especially glutamic acid, glycine and alanine; Sugai and Henrichs 1992). Some of the released substances in the autoclaved sediment were removed during the seawater rinses prior to the adsorption experiments, but the amino acid and peptide concentrations measured in rinsed sediment were still about 10 times higher than in untreated sediment. The higher concentration of glutamic acid and alanine in autoclaved sediment may have caused the relatively low adsorption of glu$_2$ and ala$_2$ at 0.01 μM compared to that in untreated sediment. Moreover, autoclaving could affect adsorption by changing the structure of some sediment organic matter (e.g., "denaturing" protein).

However, autoclaving was the only treatment tested which stopped hydrolysis without reacting with the peptides (as did formalin). As peptide adsorption varies little with concentration, the increased concentrations of amino acids and peptides resulting from autoclaving should not markedly affect adsorption.

Adsorption of ^{3}H-peptides in sediment Adsorption processes are rapid. Experiments in autoclaved sediments showed that adsorption did not increase

substantially with time after it reached apparent equilibrium at 5 hours (Figure 2.19). Comparably rapid adsorption has also been observed for amino acids on both Resurrection Bay (Henrichs and Sugai 1993) and Skan Bay (Sugai and Henrichs, in preparation) sediments. The reciprocal of k_a (Table 2.2) for lys_2 adsorption is consistent with the results shown in Figure 2.20. But for the other peptides, $1/k_a$ was much greater than 5 hours. Except in case of lys_2, the tracer model was not sensitive to adsorption, since the rate and extent of adsorption were smaller than those of hydrolysis, and thus the calculated k_a values are not accurate.

In autoclaved sediments, the adsorption of ^{3}H-peptides did not change with concentration; adsorption did not reach saturation over the concentration range from 0.01 to 333 μM (Figure 2.31). At 333 μM concentration, about 15% of lys_2 and 10% of ala_6 were adsorbed, which corresponds to concentrations of 1.06 mg-organic carbon (OC)/m^2 sediment surface area for lys_2 and 1.47 mg-OC/m^2 for ala_6 (sediment surface area values are from Mayer 1994). However, the equivalent concentration of a monolayer of organic matter coating all mineral surfaces is 0.86 mg-OC/m^2, and higher levels of adsorption within the interlamellar spaces of expandable clays were not found (Mayer 1994). Thus, there must be other adsorption sites in Skan Bay sediment in addition to the mineral components, even if mineral surfaces in sediment were responsible for part of the adsorption. Since the adsorption coefficients of peptides did not change with concentration, and since it is likely that peptide-peptide sorption occurred at high concentrations, it seems that natural sediment adsorption sites are peptide-like. Sediment organic matter could be a plausible source of such adsorption sites.

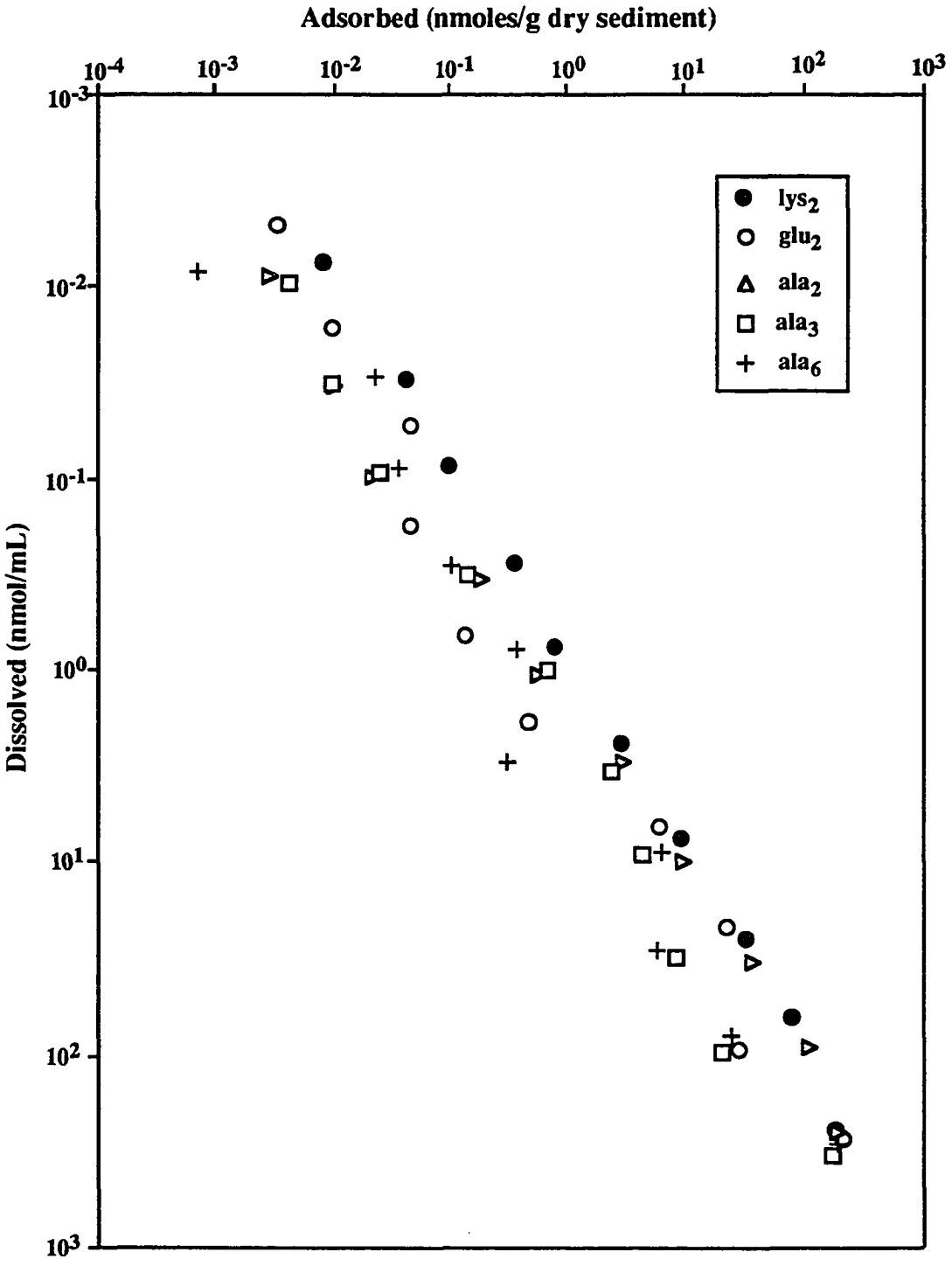


Figure 2.31. Adsorption Isotherms of ^{3}H-Peptides on Skan Bay Sediments.

The amount of peptides adsorbed (V_{ads}) per gram of dry sediment was linear with the dissolved peptide concentration in pore water (V_{diss}) on a log-log plot (Figure 2.31). The data fit the Freundlich equation:

$$V_{ads} = \kappa V_{diss}^{\frac{1}{n}}$$

If $1/n \approx 1$, κ is the partition coefficient at 1 μM peptide concentration (Wang and Lee 1993). According to the equation, 1/n is related to the curvature of the adsorption isotherm. If 1/n is less than 1, the partition coefficient decreases with increasing concentration. This situation will occur if there are limited numbers of adsorption sites, or if there are at least two different kinds of adsorption sites, with significantly different affinities for sorbate. If 1/n is greater than 1, the partition coefficient increases with concentration. Thus, the sorbate may have a greater tendency to bind with itself than with the particle surface, or there may be other substances in solution that compete for sorption sites at low concentration. If 1/n approaches 1, the units of κ are mL/g dry sediment. This situation will apply if all adsorption sites are similar and the numbers of available sites do not limit adsorption.

For the data plotted in Figure 2.31, $\kappa = 1.18$ and $1/n = 0.98$ ($r^2 = 0.998$) for ^{3}H-lys_2; $\kappa = 0.51$ and $1/n = 1.01$ ($r^2 = 0.973$) for ^{3}H-glu_2; $\kappa = 0.55$ and $1/n = 1.15$ ($r^2 = 0.993$) for ^{3}H-ala_2; $\kappa = 0.41$ and $1/n = 1.00$ ($r^2 = 0.986$) for ^{3}H-ala_3; and $\kappa = 0.33$ and $1/n = 1.06$ ($r^2 = 0.960$) for ^{3}H-ala_6. The values of 1/n for all five peptides are close to 1, which indicates the small curvature of the adsorption isotherms. Among the five ^{3}H-peptides, the κ value, i.e., adsorption extent, decreases in the order: $lys_2 > ala_2 > glu_2 > ala_3 > ala_6$. The 1/n of lys_2 is less than 1, while those of other peptides are greater than 1. This reflects the fact that adsorption of lys_2 decreases with concentration, while adsorption of the other four peptides increases with concentration (Figure 2.17). In Skan Bay sediments, there is little evidence of saturation of lys_2 adsorption sites. So, it is

likely that there is more than one kind of adsorption site for lys_2. In Skan Bay pore water, the concentrations of glutamic acid and alanine are greater than that of lysine. These substances possibly compete with chemically-similar peptides for adsorption sites. This could explain the increasing adsorption of ala_2 and glu_2 with concentration.

Lys_2 has three positively-charged amine functional groups while the other di-peptides, ala_2 and glu_2 have only one. Basicity has been found to enhance adsorption of amino acids and peptides to clay minerals and marine sediments. For example, the basic peptide L-alanyllysine was found to be adsorbed more to clays than the acidic di-peptide, L-aspglycine (Dashman 1977). Basic amino acids were two to three times more strongly adsorbed by both organic-free kaolinite and montmorillonite from 10 μM solutions in distilled water than acidic and neutral amino acids (Hedges and Hare 1987). The adsorption of positively charged organic compounds onto clays is essentially due to cation exchange and is influenced mainly by electrostatic and van der Waals forces (Wang and Lee 1990). At environmental pH values and salinity, the electrostatic interaction is generally favorable for adsorption of cations and unfavorable for adsorption of anions because most natural surfaces are negatively charged (Stumm 1987). In seawater, adsorbed organic matter gives a net negative surface charge to suspended particles (Neihoff and Loeb 1972). Also, acidic functional groups of sediment humic and fulvic acids are reported to adsorb cations (Rashid 1985). The net charge on the peptides may explain why the sediment has a relatively larger adsorption coefficient (κ) for basic lys_2 than for neutral ala_2 and acidic glu_2.

Compared with ala_2, glu_2 has two more negatively charged carboxyl groups. The difference in κ is less between ala_2 and glu_2 than between lys_2 and ala_2. Enhanced adsorption caused by additional basic functional groups is greater than any decrease caused by additional acidic functional groups. Sugai and Henrichs (in preparation) found

that the pattern of adsorption of amino acids by the sediment of Skan Bay was: lysine > alanine > glutamic acid. In this study, the adsorption of peptides has a similar pattern: lys_2 > ala_2 > glu_2. This is consistent with the generalization of Weiss (1969) that low molecular weight peptides resemble amino acids in their affinity for clay minerals.

Theoretically, van der Waals forces between surfaces and organic compounds will become stronger with increasing MW, provided there is no steric hindrance. If this force plays an important part in adsorption, adsorption should be enhanced by increased MW. Several investigations of adsorption by clay minerals (e.g., Greenland *et al.* 1962, 1965a and b; Theng 1979; Dashman 1977) have indicated that, as the MW and the chain length of glycyl peptides and other organic compounds was increased, adsorption was enhanced. However, the adsorption of alanyl peptides in this study decreased with increased chain length. Dashman and Stotzky (1984) obtained similar results for glycyl peptide adsorption on clays. They explained that the differences between their results and those of previous studies might have been due to changes in the preparation of the clay-peptide complexes, or the time that the peptides were in contact with the clays.

These are not likely explanations here, because the adsorption experiments with alanyl peptides were all done under the same conditions and the adsorption of alanyl peptides did not change over time. One explanation is that the pK_a of $-NH_3^+$ of glycyl and glycylalanyl peptides (i.e., the basicity of $-NH_2$) decreases as MW increases from amino acids to peptides (Cohn and Edsall 1943). It has been found that the adsorption of high MW organic compounds (like proteins) to artificial surfaces is related to their hydrophobicity in seawater (Kirchman *et al.* 1989). The adsorption of proteins was greater on a surface with high hydrophobicity than on a surface with low hydrophobicity. Although the hydrophobicity of ala_6 is not as high as that of the proteins examined by Kirchman *et al.* (1989), the solubility of ala_6 (less than 0.6 mM in

seawater) is much lower than that of di- and tri-peptides. However, the surfaces of the sediment particles from Skan Bay probably is more hydrophilic than the surfaces tested by Kirchman *et al.* (1989). This also may be an explanation for the low adsorption of ala_6.

Reversibility of peptide adsorption The exchange of adsorbed peptides by CsCl indicated that some of the ^{3}H-peptide adsorption, although not all of it, was by cation exchange and was reversible. Acid (HCl) extracted more adsorbed ^{3}H-peptides than ion exchange solutions in most cases. H^+ has a greater affinity for weak carboxylic acid sites than Cs^+, and thus this result suggests a role for $-COO^-$ functional groups in peptide adsorption. Since NaAc did not extract adsorbed peptides, it appears that the carboxylic groups were those of the sediment organic matter rather than the peptides. The adsorbed peptides which were not recovered by one HCl extraction could be completely recovered by several HCl extractions and acid hydrolysis (APPENDIX III, Table A.5).

Both aqueous acid and base extract fulvic acids from sediments and could have extracted peptides bound to those substances. However, NaOH was not effective in extracting adsorbed peptides (Figure 2.23). Theoretically, the pH of the 0.3 N NaOH used in this extraction was high enough to deprotonate amino groups. As most other evidence suggests that amino groups were important in peptide adsorption, and since NaOH extracts adsorbed amino acids from sediments (Henrichs and Sugai 1993), this result was unexpected. Perhaps, the NaOH solution caused a chemical reaction which removed peptides from pore water.

Effects of adsorption on decomposition Effects of adsorption on decomposition can be explained from two related perspectives. One is that the

adsorption changes the biological availability of organic materials. The other is that adsorption changes the activity of enzymes.

Comparing the hydrolysis and respiration of adsorbed and dissolved ^{3}H-peptides (Figures 2.32 and 2.33), adsorbed ^{3}H-peptides were much more resistant to hydrolysis and respiration than were the dissolved ^{3}H-peptides (except that the respiration of the adsorbed ^{3}H-glu_2 was close to that of dissolved glu_2 after 24 hours). More than 40% of the adsorbed ^{3}H-ala_2 and lys_2 remained after 50 hours and more than 70% of the adsorbed ^{3}H-glu_2 remained at 24 hours, and much of this remaining adsorbed peptide could not be extracted by acid (Figure 2.34). Thus, adsorption can decrease the rate of decomposition of peptides in sediments. Several other studies have reported that the adsorption of organic compounds by clay minerals and sediments serves to protect them from biodegradation (e.g., Christensen and Blackburn 1982; Gordon and Millero 1985; Wang and Lee 1990; Romanowski *et al.* 1991; Stewart *et al.* 1991; Sugai and Henrichs 1992).

In untreated sediments, adsorption and hydrolysis are competitive processes, which together are responsible for the rapid removal of peptides from solution. The more a peptide is hydrolyzed, the less it is adsorbed, although some of the amino acids produced by hydrolysis are adsorbed.

Most of the extracellular enzymes in sediments are bound to particles or cell surfaces (Burns 1978; Ladd 1978; Meyer-Reil 1991). It has been proposed that microbial cell-bound enzymes are mainly responsible for the decomposition of the organic material entering the sediments (Meyer-Reil 1991), althought the activity of enzymes associated with particles may be decreased compared that of free enzymes (Haska 1981; Lorenz and Wackernagel 1987; Romanowski *et al.* 1991; Stewart *et al.* 1991). The hydrolysis rate constants in extracted pore water were much less than those

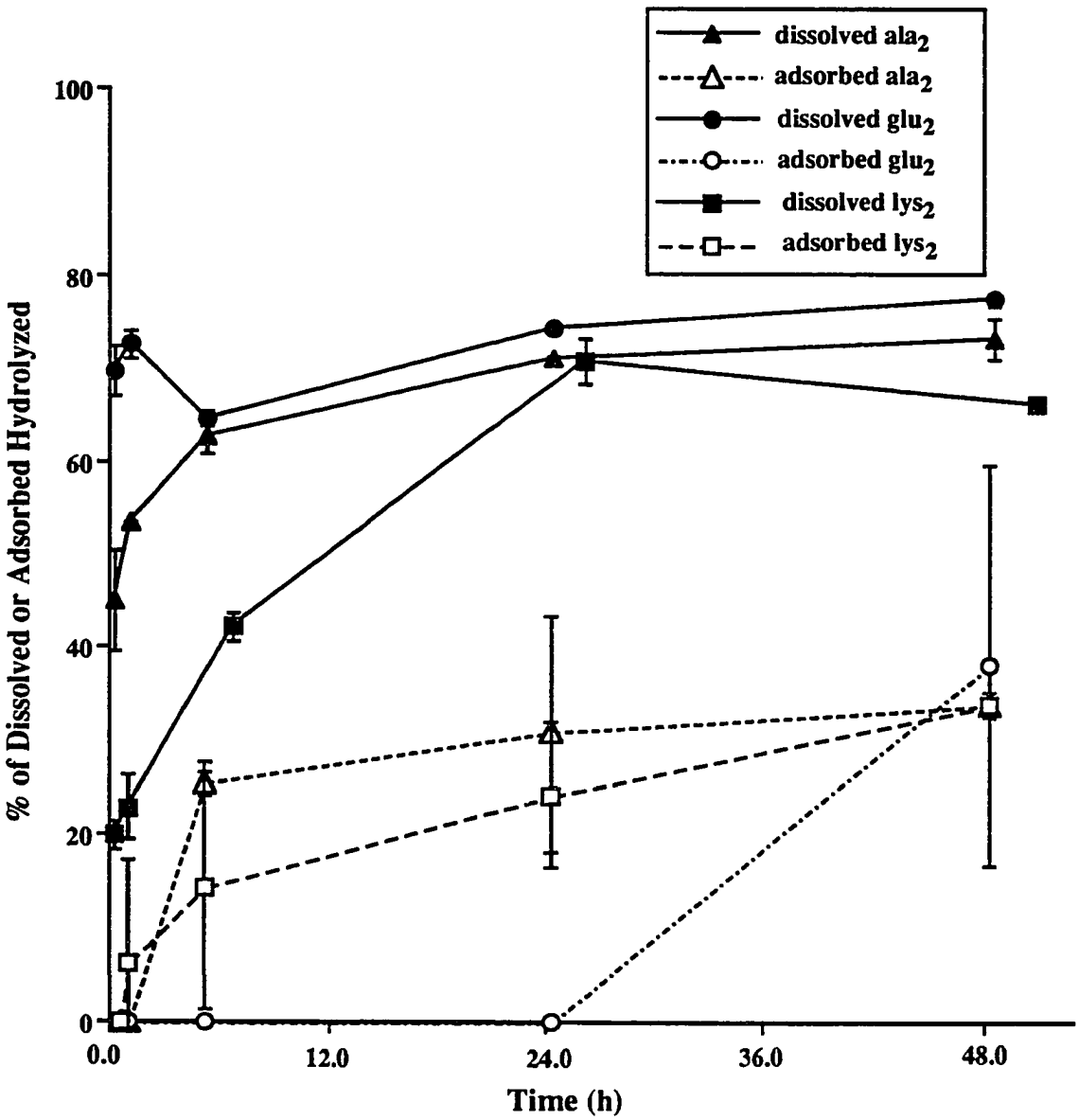


Figure 2.32. Hydrolysis of Dissolved and Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Skan Bay Sediments.

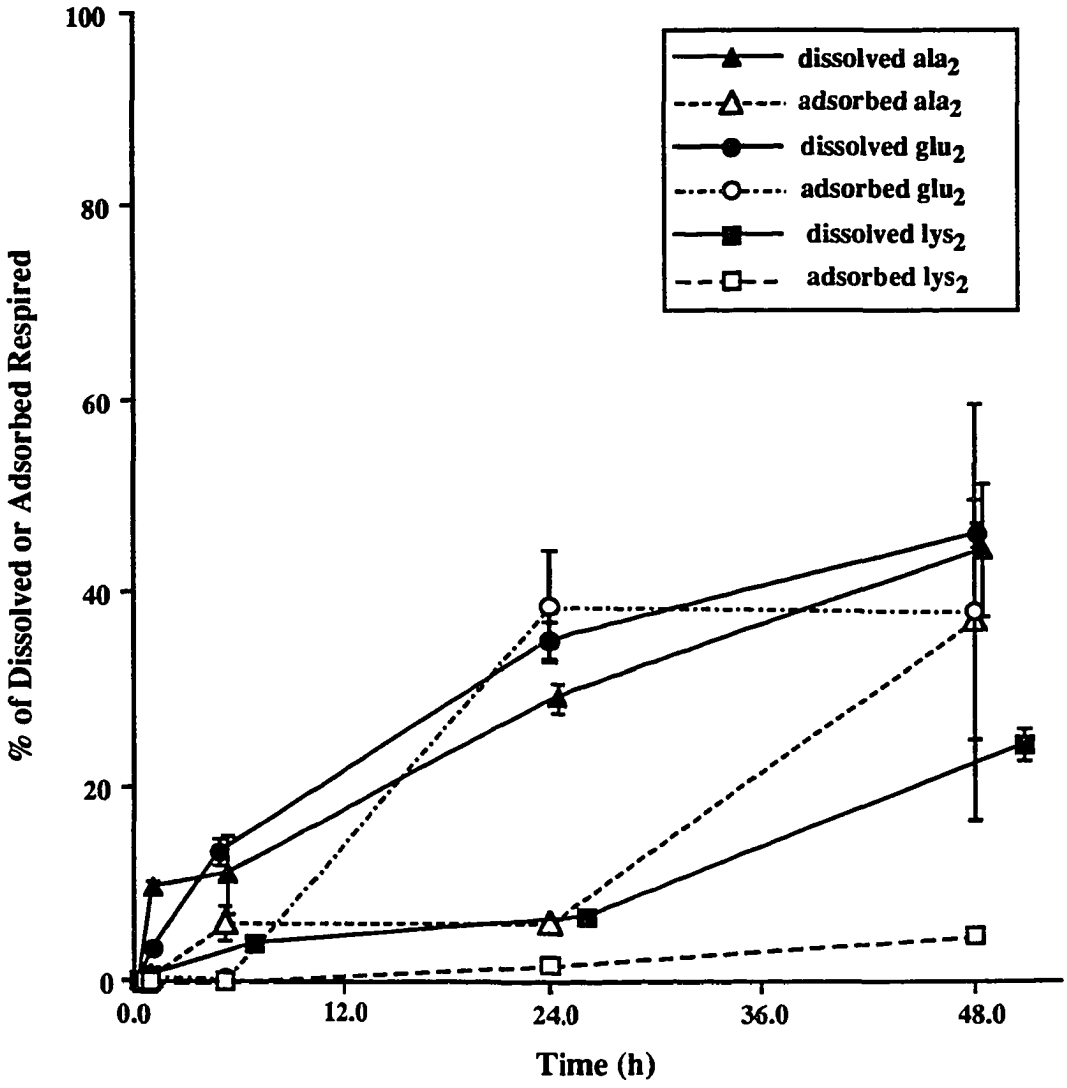


Figure 2.33. Respiration of Dissolved and Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Skan Bay Sediments.

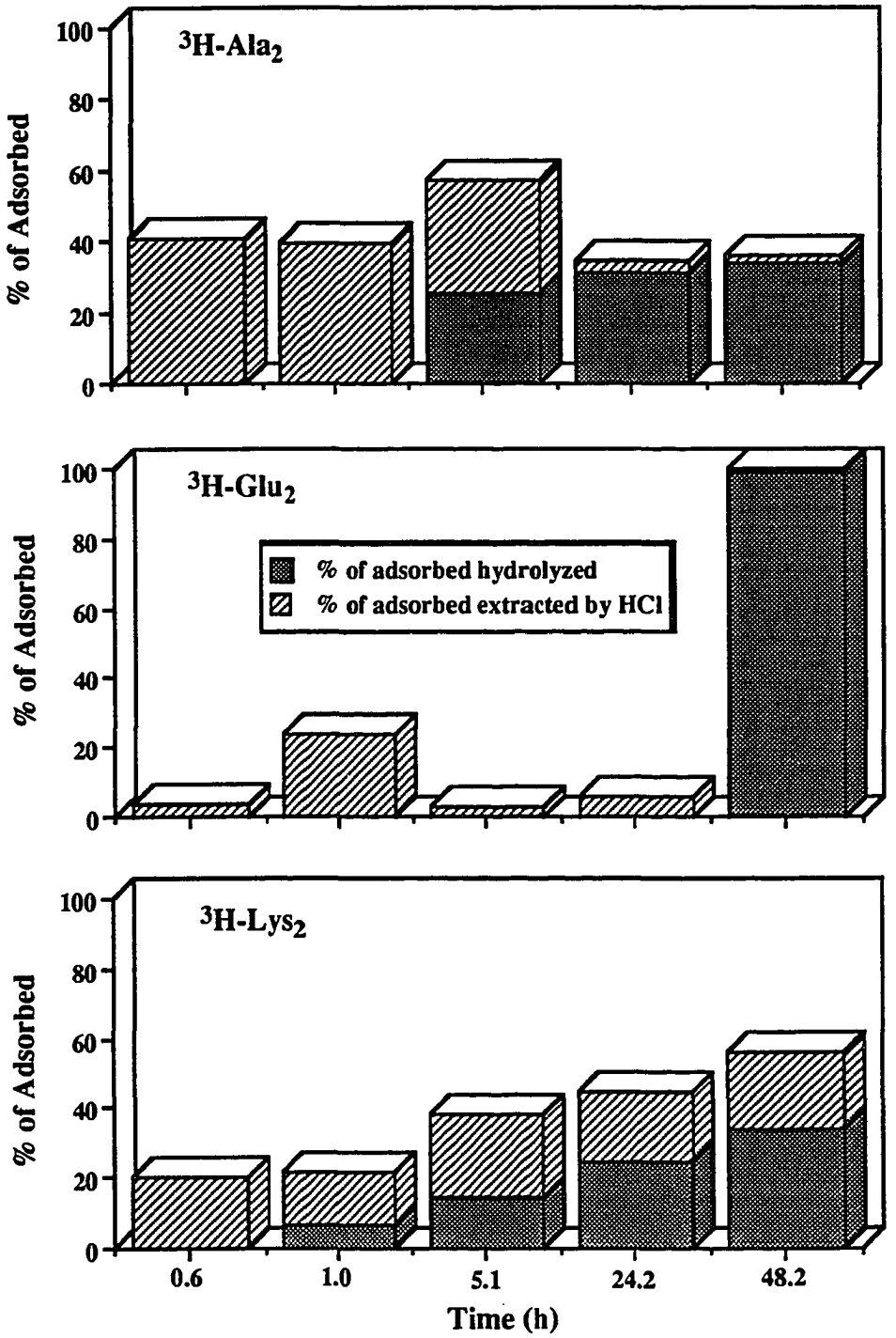


Figure 2.34. Decomposition of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Skan Bay Sediments (Autoclaved + Fresh).

in sediment from Skan Bay (Figure 2.35). The comparison of dissolved and adsorbed peptide hydrolysis and respiration rates in sediments and peptide hydrolysis rate constants in extracted pore water leads to the conclusion that cell-surface enzymes of particle-associated bacteria are probably responsible for most peptide hydrolysis. However, these enzymes are more effective in hydrolyzing dissolved peptides than adsorbed peptides, since results of this study showed that adsorption inhibits both hydrolysis and respiration. The peptides that are less adsorbed to sediments (e.g., ^{3}H-ala$_3$ and ^{3}H-ala$_6$) are more rapidly metabolized by microorganisms. Perhaps this is because adsorption prevents binding of the peptide to the active site of enzymes.

Conclusions

1. The pathway of peptide decomposition is hydrolysis followed by respiration of the released FAA. Hydrolysis rates were proportional to concentration in both pore water and sediments over a broad concentration range. Hydrolysis was the rate-limiting step in the decomposition of alanyl and glutamyl peptides in sediments.

2. All peptides were more rapidly hydrolyzed in sediment than in extracted pore water. Most enzyme activity was associated with the sediment particles.

3. The rate constants of ^{3}H-peptide hydrolysis in sediments were in the order: glu$_2$ > ala$_2$ > ala$_3$ > ala$_6$ > lys$_2$. This corresponds to the relative abundance of the corresponding amino acids in pore water.

4. Autoclaving followed by seawater rinse was the most effective killed control for measuring ^{3}H-peptide adsorption in Skan Bay sediment, and it did not affect the adsorption over time periods of less than 1 hour.

5. Adsorption isotherms of ^{3}H-peptides fitted the Freundlich equation. The adsorption partition coefficients of peptides were constant over a wide concentration

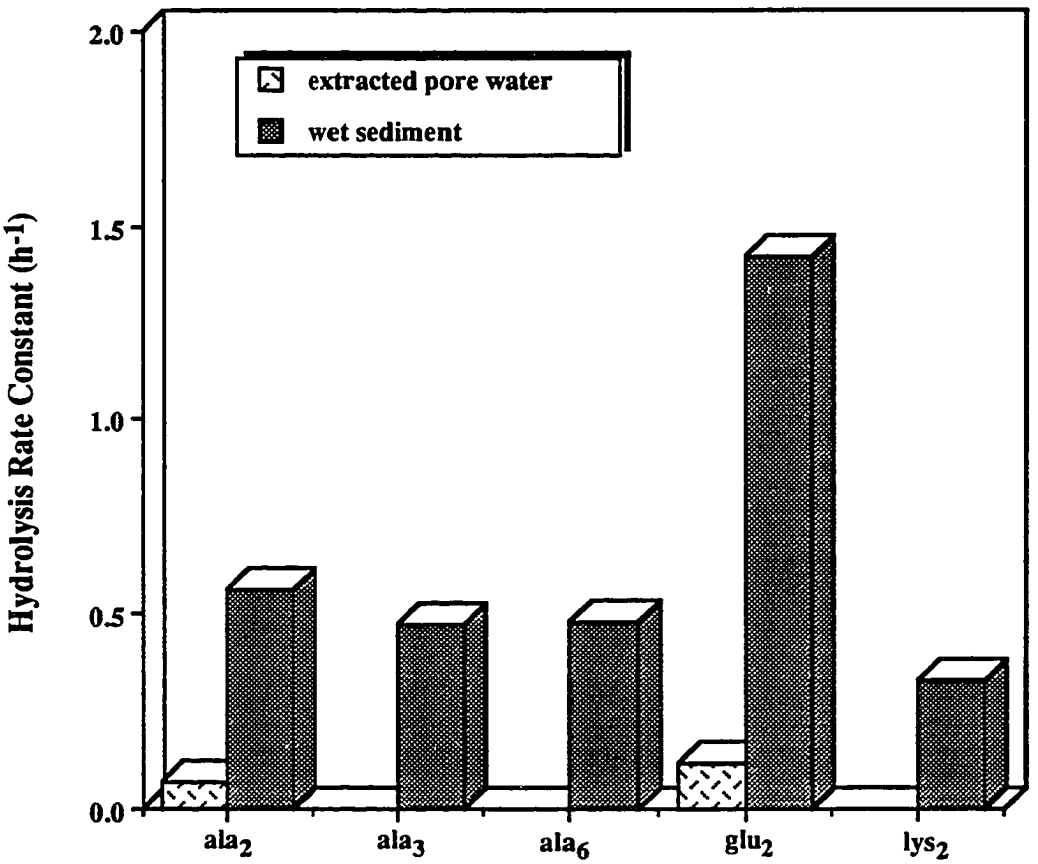


Figure 2.35. Hydrolysis Rate Constants of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Pore Water and Sediments from Skan Bay.

range, and varied in the order: 1.18 (lys_2), 1.15 (ala_2), 0.51 (glu_2), 0.41 (ala_3), 0.33 (ala_6). Saturation of adsorption sites in sediments was not observed within the solubility range of peptides in seawater.

6. The adsorption patterns of di- and tri-peptides were similar to those of free amino acids. Basicity of peptides played a more important role in enhancing adsorption than molecular weight.

7. Adsorption was most likely by interaction of peptide amino groups with the sediment organic matter carboxylic groups. However, some of the peptide adsorption was not reversible by ion exchange.

8. Adsorption of peptides onto sediment particles protected them from biodegradation.

Chapter 3: Decomposition and Adsorption of ^3H-Peptides in Resurrection Bay Sediment

Abstract

The decomposition and adsorption of C-terminal tritiated peptides (^3H-di-alanine, tri-alanine, hexa-alanine, di-lysine and di-glutamic acid) in sediments of Resurrection Bay, Alaska were studied. In pore water, ^3H-peptides were quite stable and were neither hydrolyzed nor respired in 100 hours. However, in sediments ^3H-peptides were rapidly hydrolyzed, within 1 hour. The hydrolysis rate constants of ^3H-di-alanine and di-glutamic acid were greater than those of ^3H-di-lysine and hexa-alanine. The respiration rate of ^3H-peptide to ^3H_2O varied in the order: di-alanine > tri-alanine > di-glutamic acid > hexa-alanine > di-lysine. Hydrolysis was the rate-limiting step in ^3H-peptide decomposition. For ^3H-di-alanine and di-glutamic acid, the rate of hydrolysis was much greater than that of adsorption.

The adsorption of ^3H-peptides in sediments was rapid. Adsorption of peptides increased linearly with concentration, and the partition coefficients of adsorption were: hexa-alanine > di-lysine > tri-alanine > di-alanine > di-glutamic acid. Approximately 50% of di-glutamic acid and di-lysine and 40% of di-alanine and hexa-alanine adsorption was by cation-exchange; none was by anion exchange. Some of the adsorption was irreversible, indicating that peptide adsorption in sediments may be involved in humification. Adsorbed ^3H-peptides were more resistant to decomposition than dissolved peptides.

Introduction

The factors controlling the rate and extent of organic matter decomposition in marine sediments are not fully understood (Müller and Suess 1979; Emerson 1985; Stein *et al.* 1986; Henrichs and Reeburgh 1987; Henrichs 1993). Most organic material enters marine sediments as polymeric organic compounds. One model of organic matter decomposition in sediments involves the release of soluble organic molecules due to the enzymatic hydrolysis of macromolecules by bacteria (Kim and ZoBell 1974; Krom and Sholkovitz 1977; Little *et al.* 1979; Christensen and Blackburn 1980). The released dissolved compounds are rapidly assimilated and metabolized by bacteria. Adsorption of dissolved substances to sediment could render them less available for biological decomposition and thus lead to their preservation in sediments. Organic compounds are adsorbed by marine sediments (Neihoff and Loeb 1972; Hunter 1980; Davis and Gloor 1981; Tipping and Cooke 1982). This leads to the hypothesis that adsorption is an important process affecting the sedimentary preservation of organic compounds.

There are many studies of the adsorption and decomposition of the dissolved intermediates (e.g., amino acids or low-molecular-weight carboxylic acids) of organic matter decomposition in marine sediments (e.g., Crill and Martens 1986; Burdige and Martens 1990; Sugai and Henrichs 1992). Decomposition and adsorption of amino acids in sediments of Resurrection Bay, Alaska have been studied by Doyle (1988), McDaniel (1989), Sugai and Henrichs (1992), and Henrichs and Sugai (1993). Free amino acids were lost from the dissolved pool by both bacterial uptake and adsorption to sediment particles. Adsorption was the dominant removal process for the basic amino acid lysine and was responsible for about half of the rapid, initial glutamic acid and alanine loss from solution. Most amino acids were adsorbed irreversibly. A melanoidin-type reaction with reactive functional groups of sediment organic matter may have been one

adsorption mechanism. Amino acid adsorption could produce a significant amount of the total refractory sediment organic nitrogen.

A large proportion of the organic carbon and nitrogen in coastal sediments has been identified as hydrolyzable amino acids. However, there are only a few reports of the decomposition rates of peptide analogs and proteinaceous substrates (e.g., King 1986; Meyer-Reil 1987, 1991) or studies of the adsorption of peptides and proteins on clay minerals and other solid materials (Dashman 1977; Kirchman *et al.* 1989; Taylor *et al.* 1994). Extending the prior studies of amino acids to peptides could potentially better-explain the preservation of bound amino acids in sediments.

In this study, I investigated the decomposition and adsorption of five C-terminal ^{3}H-labeled peptides in sediments of Resurrection Bay, Alaska. The selected peptides represent three different groups based on their side-chain properties: basic di-lysine (lys_2), acidic di-glutamic acid (glu_2) and aliphatic di-alanine (ala_2), tri-alanine (ala_3) and hexa-alanine (ala_6). These peptides are composed of the amino acids lysine, glutamic acid and alanine that have been previously studied in this sediment (Sugai and Henrichs 1992; Henrichs and Sugai 1993). The comparison between peptides and free amino acids could yield information on how molecular weight and functional groups affect the decomposition and adsorption of organic matter in sediment. My objectives were to delineate the decomposition pathway of peptides, to estimate the decomposition rates of peptides and amino acids derived from them, and to determine the effects of adsorption on peptide decomposition.

Materials and Methods

Study site The sediments examined were collected in May 1993. The sampling site was Thumb Cove (60°00.4'N, 149°18.3'W), Resurrection Bay, a subarctic fjord in

south-central Alaska surrounded by steep mountains (Figure 3.1). Samples were taken from a water depth of 60 m. The sediments are largely detrital silt, and contain 0.7% total organic carbon (TOC). They are oxic to suboxic, with a light gray or brownish-gray color at the surface. The bottom water is always oxic.

Sampling on board ship Sediment samples were collected with a box corer. After removing the overlying water, plates with holes at about 4.5 cm depth were inserted vertically into the mud. Subsamples were collected by inserting 10 or 12 mL plastic or glass syringes horizontally into the plate holes. After filling, the plastic syringes were sealed with rubber stoppers and glass syringes were sealed with Teflon-faced silicone septa and aluminum crimp seals. Sediment samples for adsorption experiments were collected from box cores by manually scooping the 4.5 - 6 cm layer into mason jars. The mason jars were sealed and stored in an incubator for experiments conducted later in our Fairbanks laboratory.

Peptide solutions The tritiated (^{3}H-) peptides, di-glutamic acid (glu_2), di-alanine (ala_2), tri-alanine (ala_3), hexa-alanine (ala_6) and di-lysine (lys_2), used in this study were synthesized by extending the C-terminal end of commercially available non-labeled peptides by azide coupling with tritiated amino acids (i.e., [3, 4-^{3}H] glutamic acid, [2, 3-^{3}H] alanine, and [4, 5-^{3}H] lysine) (Bodanszky and Bodanszky 1984a, b). The labeled peptides were separated and purified using thin-layer chromatography and using reversed-phase high performance liquid chromatography (HPLC) (Iskandarani and Pietrzyk 1981). In each ^{3}H-peptide product, 60 to 86% of tritium activity was ^{3}H-peptide and the remainder was the corresponding ^{3}H-free amino acid. The specific activities of synthesized ^{3}H-glu_2, ^{3}H-ala_2, ^{3}H-ala_3, ^{3}H-ala_6 and ^{3}H-lys_2 were 152.1, 399.5, 276.2, 133.1 and 246.8 mCi/mg, respectively. Only the decomposition of the C-terminal residue can be monitored using these peptides.

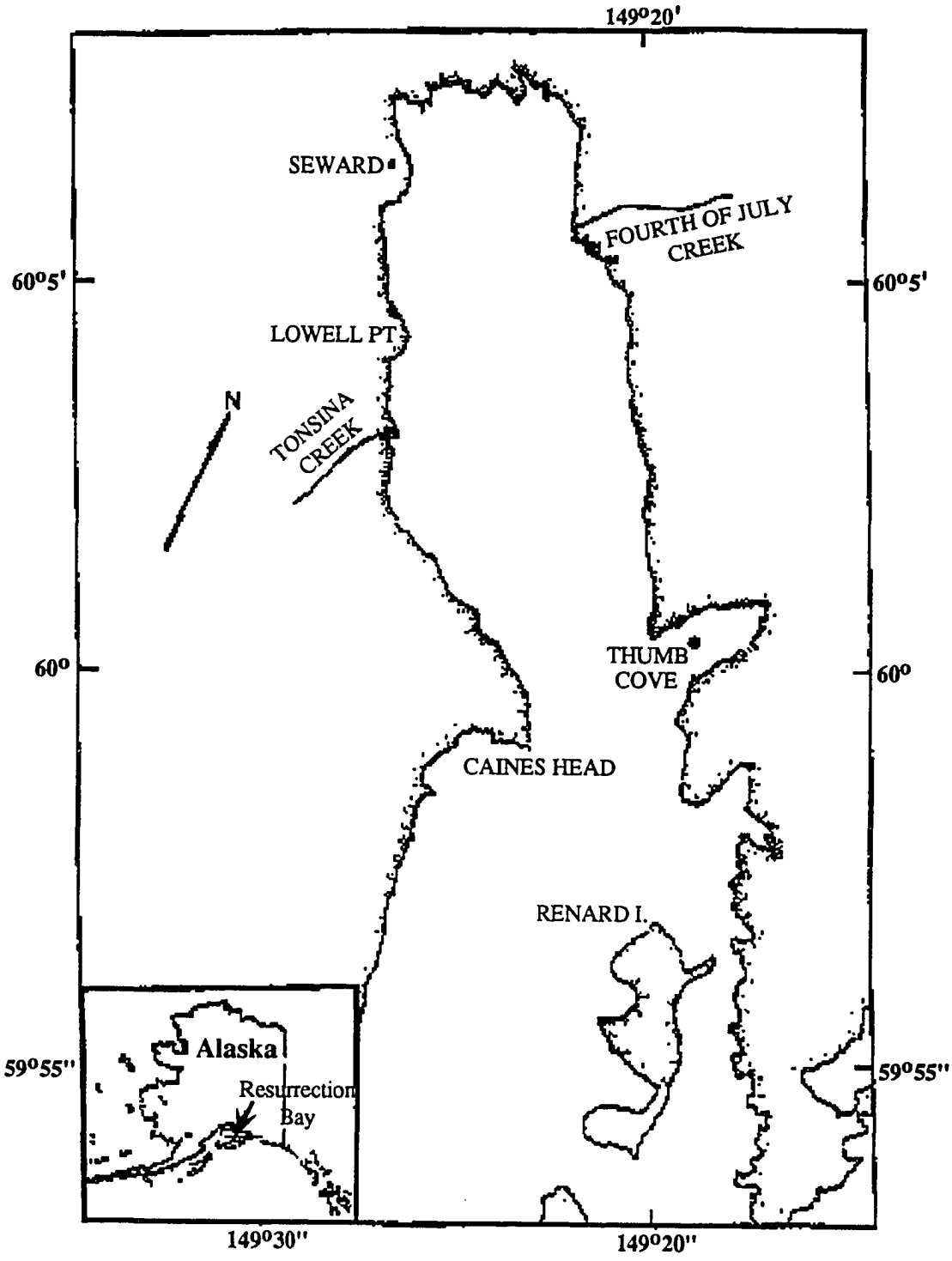


Figure 3.1. Map of Resurrection Bay.

Non-labeled peptides (Sigma Chemical Company) were dissolved in filtered (Whatman GF/F) seawater to make solutions at the concentrations needed for experiments. ^{3}H-peptides dissolved in glass-distilled water (less than 300 µL) were added to non-labeled peptide solutions at a concentration of 10^4 dpm/mL.

High Performance Liquid Chromatography The separation of ^{3}H-peptides from ^{3}H-free amino acids and $^{3}H_2O$, as well as the measurement of peptide concentrations, was conducted using reversed-phase HPLC. The analytical columns were Lichrosorb RP-18 (250 mm x 4.0 mm, 10 µM particle size) and Econosphere C18 (250 mm x 4.6 mm, 5 µM particle size).

For the separation of ^{3}H-fractions, one to 1.5 mL of solution was injected after filtration through a 0.2 µm Nuclepore filter. The mobile phases were methanol and 0.01% acetic acid adjusted with 6 N HCl to pH 1.4, or methanol and 0.03% acetic acid (Iskandarani and Pietrzyk 1981; Mant and Hodges 1991). Fractions of the eluate containing tritium were collected and ^{3}H-peptides were separated from related ^{3}H-free amino acids (FAA) by elution time. Ten mL of liquid scintillation solution (UniverSol, ICN Biomedicals, Inc.) was added to each collected fraction. The radioactivity was determined by liquid scintillation counting (Beckman model LS 3801), after waiting at least several hours to let any chemiluminescence die out.

Peptide concentrations were determined by precolumn fluorescence derivatization of peptides with *o*-phthaldialdehyde/α-mercaptoethanol (OPT) reagent (Lindroth and Mopper 1979; Jones *et al.* 1981). Fifty mg of *o*-phthaldialdehyde and 100 mg dodecyl sulfate-mercaptoethanol were dissolved in 5 mL methanol and allowed to "age" for 3 to 4 days. The peptide solution was reacted with 50 µL of OPT reagent and 100 µL of 0.4 M sodium borate buffer at room temperature for exactly 2 minutes. Samples were filtered through 0.4 µm GF/F filter before derivatization. For samples

which had a large amount of ammonia, the filtrate was stripped of ammonia at 80°C for 30 minutes before derivatization. Phosphate buffer (0.02 M, pH 6.8) and methanol or phosphate buffer and acetonitrile were employed as mobile phases. The detector was a Kratos Model FS 950 Fluoromat. Under the chromatographic conditions employed, the five peptides studied were separated from the free amino acids which were usually present in pore water.

Decomposition experiments For experiments in pore water, fresh sediment was transferred from a plastic sampling syringe to a centrifuge tube on board ship. For some experiments in the Fairbanks laboratory, the transfer of sediment from mason jars to centrifuge tubes was done under nitrogen. After centrifugation at -1°C for 10 minutes, the pore water was filtered through a 0.2 μm Nuclepore filter. The filtered pore water was degassed by bubbling with argon or nitrogen for 20 minutes. ^{3}H-peptide solutions were added and mixed with pore water in a volume ratio of 1:2. The incubation was stopped by freezing the mixture, and it took about 15 minutes for 10 mL of pore water to freeze. For the short-term incubations (time in minutes), the incubation was stopped by boiling in water for 60 sec, then the mixture was cooled in an ice-bath for another 60 seconds before analysis by HPLC.

For the study of decomposition in sediments, sediment samples (10 to 12 mL) were transferred (for fresh sediment, from a plastic syringe; for autoclave-treated sediment, from the mason jar used for autoclaving) to a 50 mL centrifuge tube under argon gas. Three mL of a 0.1 μM ^{3}H-peptide standard solution was added and mixed for 1 minute, effectively diluting the peptide solution to about 0.03 μM with the pore water, then placed in an incubator at 2 to 4°C for 0, 1, 5, 10, 24, and 48 hours. After incubation, the slurry was centrifuged at -1°C for 10 minute and the supernatant was filtered through a 0.4 μm GF/F filter. The sediment pellet was extracted with 6 mL of 0.8 N HCl solution

for 2 minutes (the final concentration in extracts was 0.3 N) and centrifuged. One mL of the filtered acid extract (AE) was mixed with 10 mL of scintillation fluid and allowed to sit at least overnight before scintillation counting. The rest of the filtrate was frozen at -30°C and analyzed by HPLC within a month after the cruise. For the short-term incubations, the filtrate (in vials) was placed in boiling water for 60 seconds to stop any further bacterial activity, then quickly cooled to room temperature and analyzed by HPLC.

Killed controls Autoclaving was used as a means of eliminating biological activity. Sediment sealed in a mason jar (250 or 500 mL) was heated in a pressure cooker at 15 p.s.i. for 2 hours. The autoclaved sediment was not rinsed with filtered seawater, because the Resurrection Bay sediment was very sticky and difficult to rinse. Experiments with Skan Bay sediment showed that rinsing decreased the concentration of organic substances which are introduced into pore water by autoclaving. However, rinsing had little effect on peptide adsorption by Resurrection Bay sediment.

Adsorption experiments The adsorption experiments were conducted on fresh sediments using both nonlabeled and labeled peptides. The concentrations of the standard solutions were 0, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 and 1,000 μM. For ala_6, the 1,000 μM solution was omitted because of its low solubility in seawater. During the mixing and centrifuging procedure, the sediment samples were in contact with the peptide solutions for 15 to 18 minutes (APPENDIX IV, Figure A.1a).

Exchange and extraction experiments Both exchange and extraction solutions in these experiments were prepared with seawater which had been filtered through 0.2 μm Nuclepore filters. Seawater, sodium acetate and cesium chloride (CsCl) solutions were used to exchange the adsorbed peptides. 0.8 N HCl (acid) and 1N NaOH (base) were used as extraction solutions (APPENDIX IV, Figure A.1b). Six mL of

exchange or extraction solutions were added to each 10 or 12 mL of sediment. For the HPLC analysis of the acidic and basic extraction solutions, the acidic extracts were neutralized with 6 N NaOH solution, and basic extracts were neutralized with 6 N HCl and filtered before injection.

Pretreatment experiments Sediments were mixed with seawater, 1 M CsCl, or 1 M sodium citrate solutions and allowed to react for 1 hour, then centrifuged to separate them from the pretreatment solutions. Filtered seawater was added to the pretreated sediment pellet to compensate for the pore water loss during the treatment. Then ^{3}H-peptide solutions were added to the pretreated sediments, as described for adsorption experiments. After adsorption, the solutions were separated from sediments by centrifugation, and then the sediment residues were extracted with 5 mL 0.8 N HCl for 2 minutes. The peptide solutions from the sodium citrate pretreated sediments were treated with 1 M $BaCl_2$ to precipitate the citrate before HPLC analysis.

Decomposition of adsorbed peptides This experiment examined how rapidly-adsorbed ^{3}H-peptides (lys_2 and ala_6) were decomposed biologically. Three mL of 0.1 μM ^{3}H-peptide solutions were first incubated in 10 to 12 mL of autoclaved sediment for 1 hour to ensure that ^{3}H-peptide was adsorbed. The dissolved peptides remaining in pore water retained by the sediment pellet were removed by rinsing three times with 5 mL filtered seawater. The rinsed sediment particles containing adsorbed peptides were mixed with 10 to 12 mL of fresh sediment and 2 mL filtered seawater under argon gas, and then incubated at 2 to 4°C for 0, 1, 5, 10, 24, and 48 hours (APPENDIX IV, Figure A.1c). The mixing efficiency of autoclaved and fresh sediments was 65% which was measured by adding $^{3}H_2O$ into blank samples without added radiolabeled peptides. This fraction was considered in the subsequent calculations.

Calculation of parameters The decomposition of ^{3}H-peptides was evaluated as the percentage of added peptide activity which was recovered as ^3H_2O and ^{3}H-FAA. The rates of ^{3}H-peptide hydrolysis and respiration were calculated in units of nanomoles of ^{3}H-peptide per cm^3 of pore water (pw) or sediment per day.

In sediments,

$$\% \text{ of } ^3\text{H-peptide respiration} =$$

$$100x \frac{[^3H_2O \text{ in pw}] - [^3H_2O \text{ in added solution}] - [^3H_2O \text{ respired from added } ^3\text{H-FAA}]}{[^3\text{H-peptide in added solution}]} \quad \text{---[1]}$$

^3H_2O respired from added ^{3}H-FAA was calculated from the mineralization rates of free amino acids in Resurrection Bay sediments (Sugai and Henrichs 1992).

Because 10 to 30% of the added activity was ^{3}H-FAA, the fraction of the ^{3}H-FAA in the added solution must be considered in calculating the hydrolysis of ^{3}H-peptide. Assuming all the ^3H_2O in pore water is produced by the respiration of ^{3}H-FAA, the actual hydrolysis should include not only the ^{3}H-FAA remaining in pore water, but also the ^{3}H-FAA that was adsorbed to sediments or respired to ^3H_2O (Eq. [2]). The fraction ^{3}H-FAA adsorbed was estimated from the ^{3}H-FAA in the measured acid extract (Henrichs and Sugai 1993).

$$\% \text{ of hydrolysis} =$$

$$100x \frac{[^3H_2O \text{ produced}]R_1 + [^3\text{H-FAA in pw}] + [^3\text{H-FAA in AE}]R_2 - [^3\text{H-FAA added}]}{[^3\text{H-peptide added}]} \quad \text{----[2]}$$

[^3H_2O produced] is the [^3H_2O in pw] corrected for the [^3H_2O in added solution], and [^{3}H-FAA in AE] is the ^{3}H-FAA in the acid extract. R_1 is an estimate of the following ratio for the amino acid, when taken up by the sediment bacteria:

$$R_1 = \frac{[^3H_2O \text{ produced}] + [\text{biomass produced}]}{[^3H_2O \text{ produced}]} = 1 + \frac{[\text{biomass produced}]}{[^3H_2O \text{ produced}]}$$

$$= 1 + \frac{[^3\text{H added}] - [^3\text{H in pw}] - [^3\text{H in AE}]}{[^3H_2O \text{ produced}]}$$

R_2 is an estimate of the irreversible to reversible adsorption ratio of ^{3}H-FAA when reversibly adsorbed FAA are those extracted by acid solution:

$$R_2 = \frac{[\text{irreversible}] + [\text{reversible}]}{[\text{reversible}]}$$

R_2 was calculated from the data of Henrichs and Sugai (1993).

The fraction of ^{3}H-peptide adsorbed and the fraction of the adsorbed ^{3}H-peptide exchanged or extracted were calculated using the following equations:

$$\% \text{ of adsorption} = 100 - \% \text{ of hydrolysis} - \% \text{ remaining in pore water} \quad \text{----[3]}$$

$$\% \text{ of adsorbed exchanged} = 100 \times \frac{\% \text{ of exchange}}{\% \text{ of adsorption}} \quad \text{----[4]}$$

$$\% \text{ of adsorbed extracted} = 100 \times \frac{\% \text{ of extraction}}{\% \text{ of adsorption}} \quad \text{----[5]}$$

Because McDaniel (1989) found that the respiration of ^{3}H-FAA in Resurrection Bay pore water was very slow compared to the incubation times used in this study, the amount of peptide respiration can be calculated using Eq. [1]. The amount of hydrolysis at each incubation time can be calculated using Eq. [6]:

$$\% \text{ of hydrolysis} = 100 \times \frac{[^3\text{H-FAA in pw}] - [^3\text{H-FAA added}]}{[^3\text{H-peptide added}]} \quad \text{----[6]}$$

Results

Decomposition of ^{3}H-peptides in pore water ^{3}H-peptides (ala_2, ala_6, glu_2 and lys_2) were quite stable in pore water (Figure 3.2). Peptides were neither hydrolyzed nor respired during the first 80 hours of incubation.

Decomposition of ^{3}H-peptides in sediments ^{3}H-peptides were decomposed much more rapidly in sediments than in pore water. At 0.03 μM concentration, ^{3}H-peptides were substantially hydrolyzed after 1 hour (Figure 3.3), and hydrolysis reached a maximum at about 5 hours. After about 12 to 15 minutes, 14 to 20% of the added ^{3}H-ala_2, glu_2 and lys_2 was hydrolyzed but only 3% of the added ^{3}H-ala_6. After 1 hour, hydrolysis increased to 65 to 70% of added activity for ^{3}H-ala_2 and glu_2, but to only 18 to 25% for ^{3}H-lys_2 and ala_6. After 5 hours, hydrolysis reached a maximum of about 90% for ^{3}H-ala_2, 70% for ^{3}H-glu_2 and lys_2, and 60% for ^{3}H-ala_6. The extent of ^{3}H-peptide hydrolysis was in the order: $ala_2 > glu_2 \geq lys_2 > ala_6$.

The respiration of added ^{3}H-peptides was very low at 15 minutes (Figure 3.4). After 10 hours, ^{3}H-ala_2 and ala_6 respiration increased greatly, and it reached a maximum of 65% and 40%, respectively. ^{3}H-lys_2 respiration reached a maximum of about 25% after 24 hours. The amount of ^{3}H-glu_2 respiration increased steadily with time to 45% after 48 hours. The extent of respiration was in the order: $ala_2 > ala_6 \geq glu_2 > lys_2$.

Figure 3.5 shows the changes over time in added ^{3}H-peptide, ^{3}H-FAA, and ^3H_2O in pore water and acid extracts during the incubation period. The added ^{3}H-peptides were removed rapidly from the dissolved pool, either by hydrolysis or by adsorption. Almost all of the added ^{3}H-ala_2, glu_2 and lys_2 had disappeared from the dissolved pool after 5 hours, while 15 % of the added ^{3}H-ala_6 remained in pore water at 48 hours. No ^{3}H-ala_2 was extractable in HCl solution at any time, while less than 10% of ^{3}H-ala_6 was acid-extractable. The acid extraction of ^{3}H-glu_2 decreased from 10 to

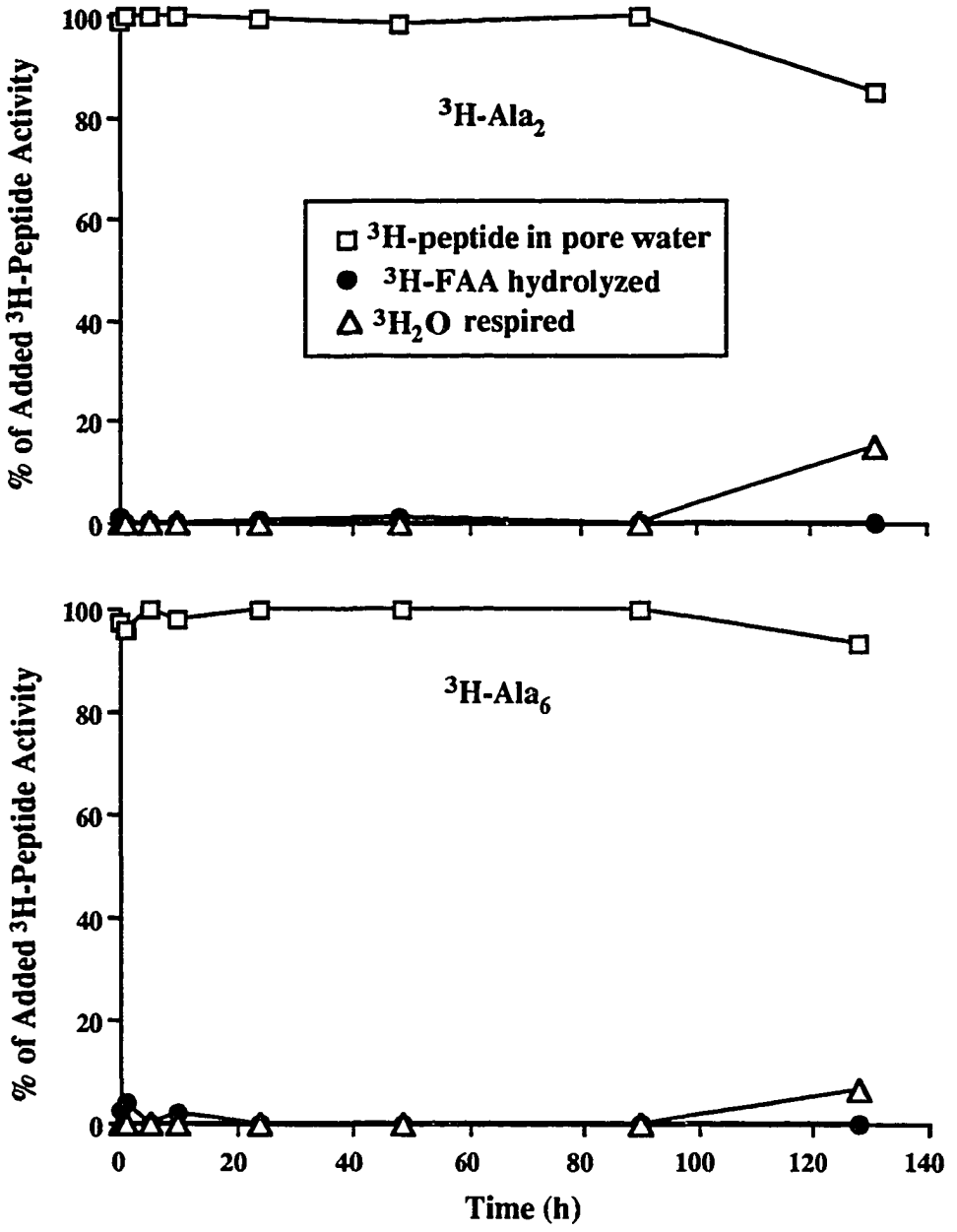


Figure 3.2. Decomposition of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Resurrection Bay Pore Water.

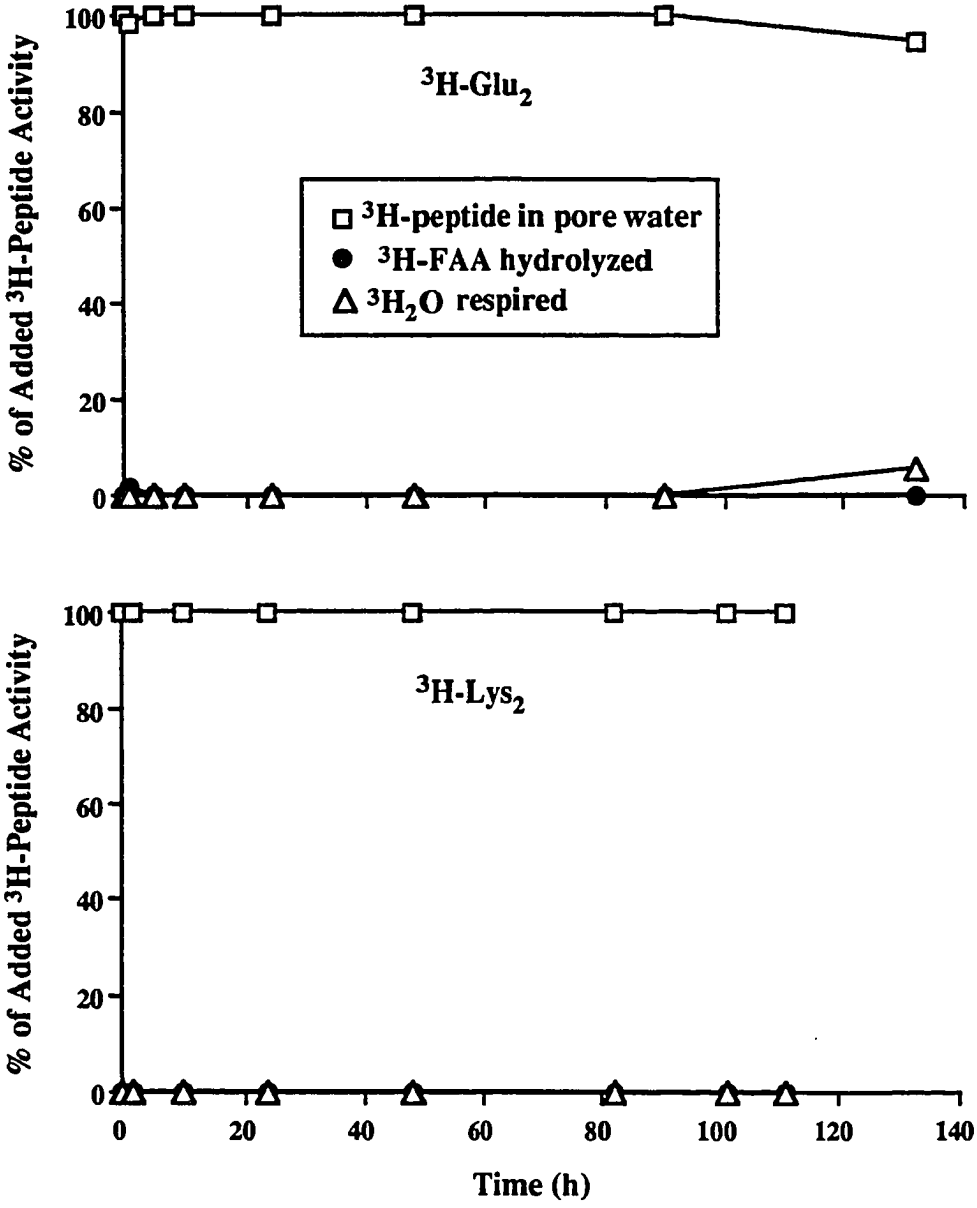


Figure 3.2. (continued)

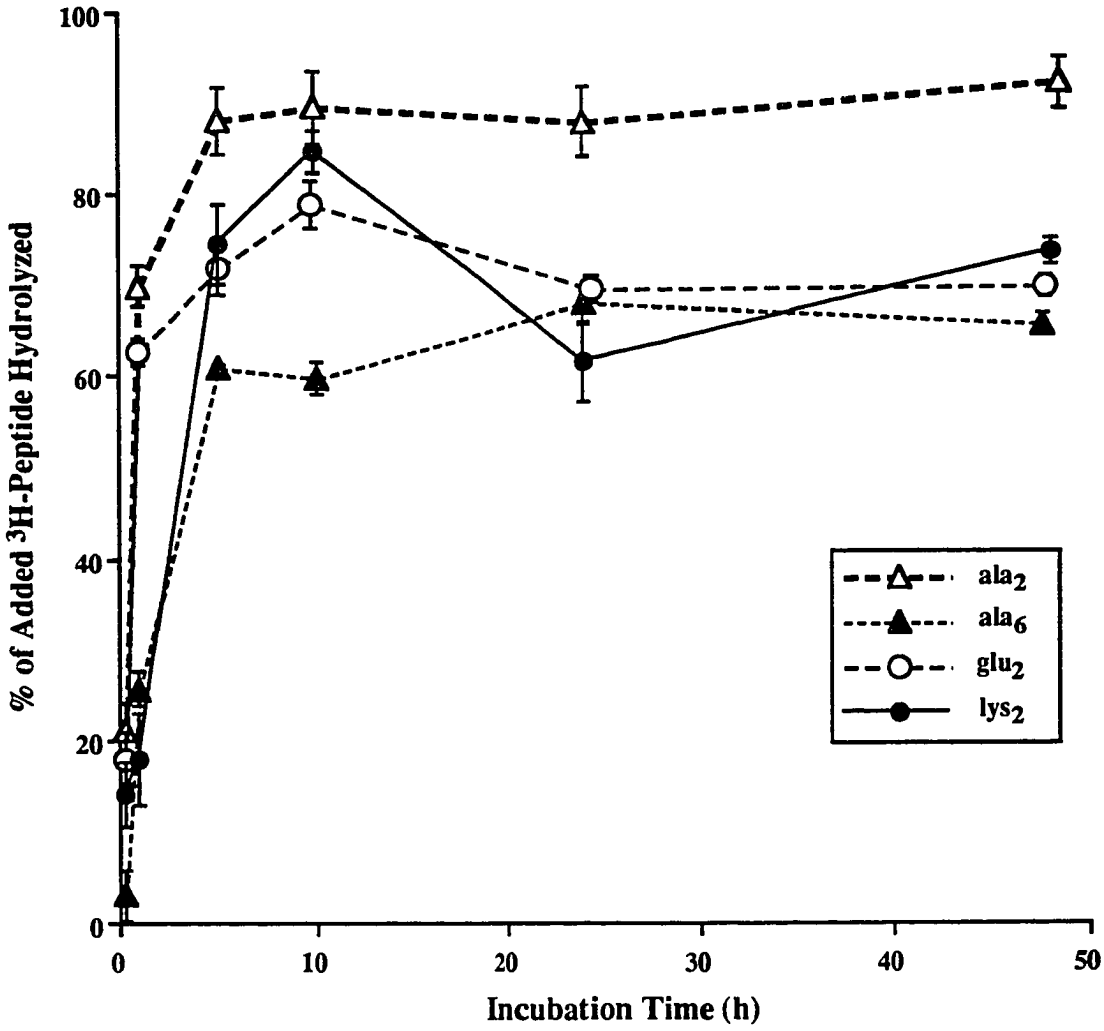


Figure 3.3. Hydrolysis of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Resurrection Bay Sediments.

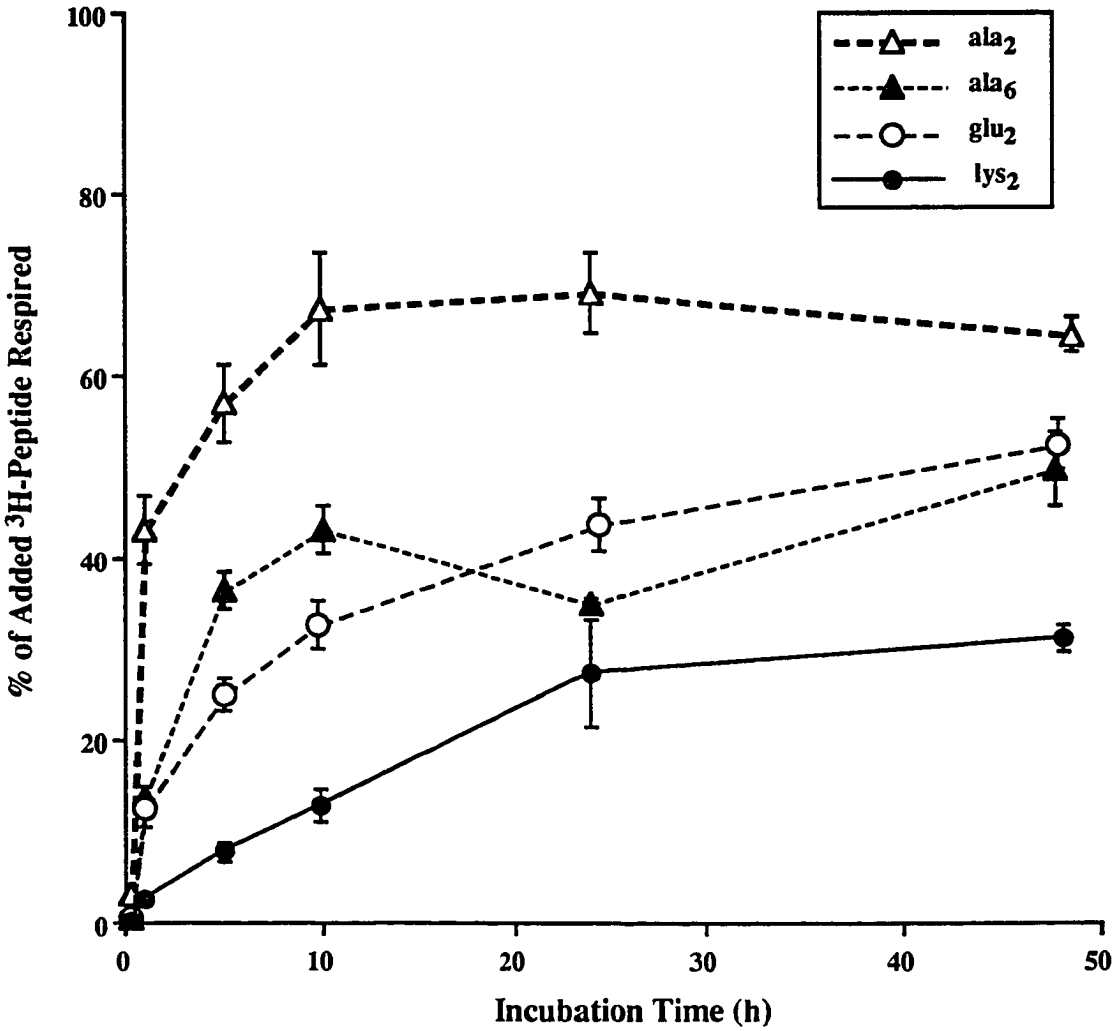


Figure 3.4. Respiration of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Resurrection Bay Sediments.

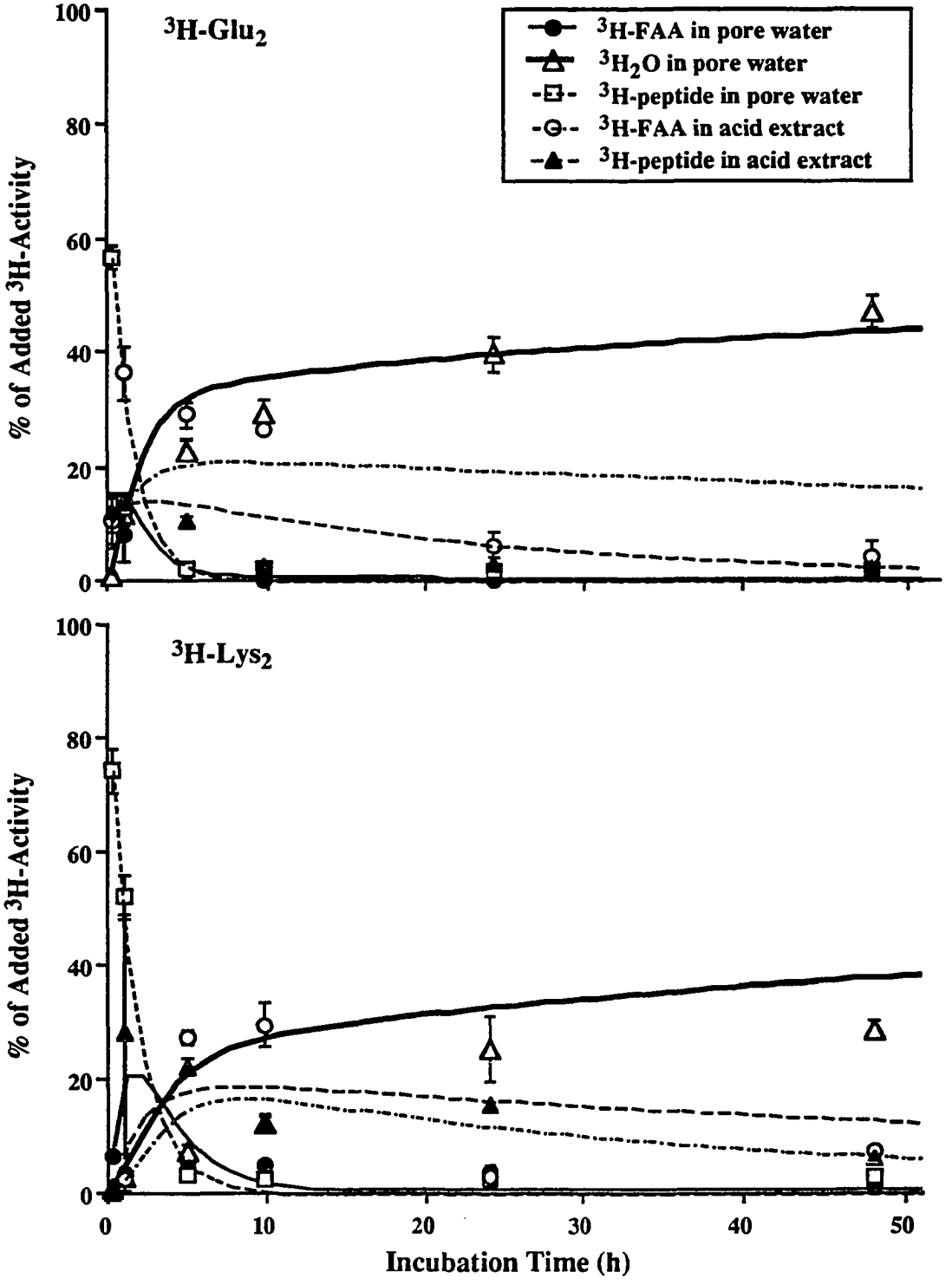


Figure 3.5. Decomposition of ^{3}H-Peptides in Resurrection Bay Sediments. Lines drawn were calculated using the tracer model described in the text and parameters given in Table 3.1.

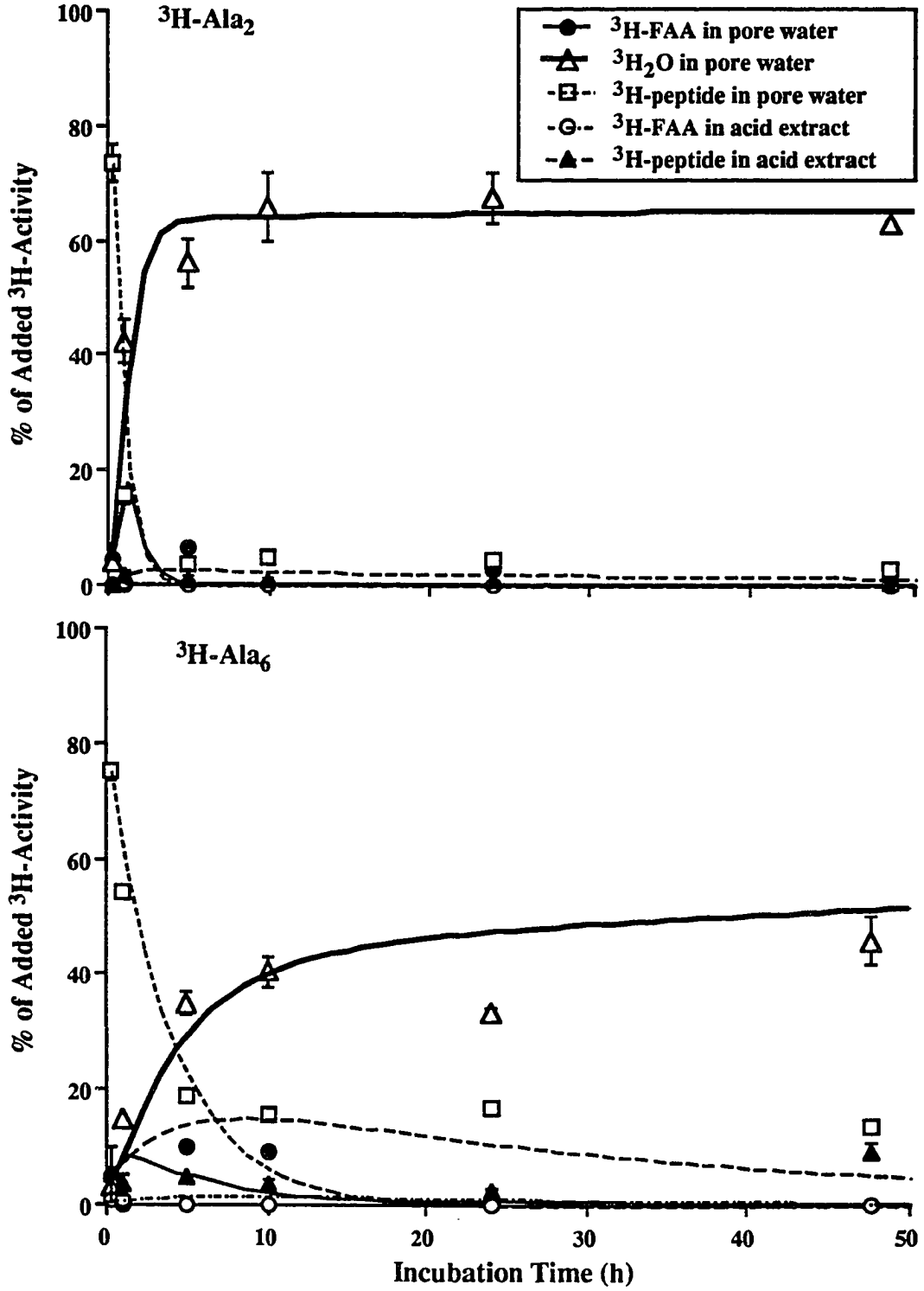


Figure 3.5. (continued)

14% during the first 5 hours to less than 4% after 10 hours. The acid extraction of ^{3}H-lys_2 increased from 0% at 15 minutes to about 25% at 1 hour, and then it decreased gradually after 5 hours. The amount of ^{3}H-FAA in pore water (from hydrolysis and the added solution) was low, less than 10% of added activity at any time. The amount of ^{3}H-FAA in the acid extract was nearly zero for ^{3}H-ala_2 and ala_6. For ^{3}H-glu_2 and lys_2, acid-extractable ^{3}H-FAA were 25 to 36% between 1 and 10 hours, but less than 10% at other time points. Corresponding to the decrease in ^{3}H-peptides in pore water, the ^3H_2O increased over time (Figure 3.4).

In the concentration range from 0 to 333 μM, the rate of ^{3}H-peptide hydrolysis (except for glu_2 hydrolysis) during the first 18 minutes increased with concentration, especially at concentrations greater than 0.03 μM (Figure 3.6). The increase was greater for ^{3}H-ala_2 and ala_3 than for ^{3}H-ala_6 and lys_2. However, the relative extent of ^{3}H-glu_2 hydrolysis decreased slightly with concentration. Thus, at low concentrations (< 0.03 μM), the rate of ^{3}H-peptide hydrolysis was in the order $glu_2 \geq ala_2 > lys_2 > ala_6 > ala_3$, while at higher concentrations, the order became $ala_2 \geq ala_3 > lys_2 \geq ala_6 \geq glu_2$. At concentrations less than 0.03 μM, there was little or no respiration of ^{3}H-peptides during the first 18 minutes (Figure 3.7). At higher concentrations, only ^{3}H-ala_2 and ala_3 were respired, a maximum of 20% of ^{3}H-ala_2 and 8% of ^{3}H-ala_3.

The decomposition of ^{3}H-peptides in stored sediments (collected in July, 1989) was studied in March 1992. In stored sediments, the amounts of ^{3}H-glu_2, ala_2 and ala_3 hydrolysis at 0.03 and 3.33 μM initial concentrations were much greater than in fresh sediments, as was the hydrolysis of ^{3}H-ala_6 at 0.03 μM (Figure 3.8). However, the hydrolysis of ^{3}H-lys_2 was similar in both stored and fresh sediments. Also, the extent of ^{3}H-peptide respiration was similar in fresh and stored sediments (Figure 3.9).

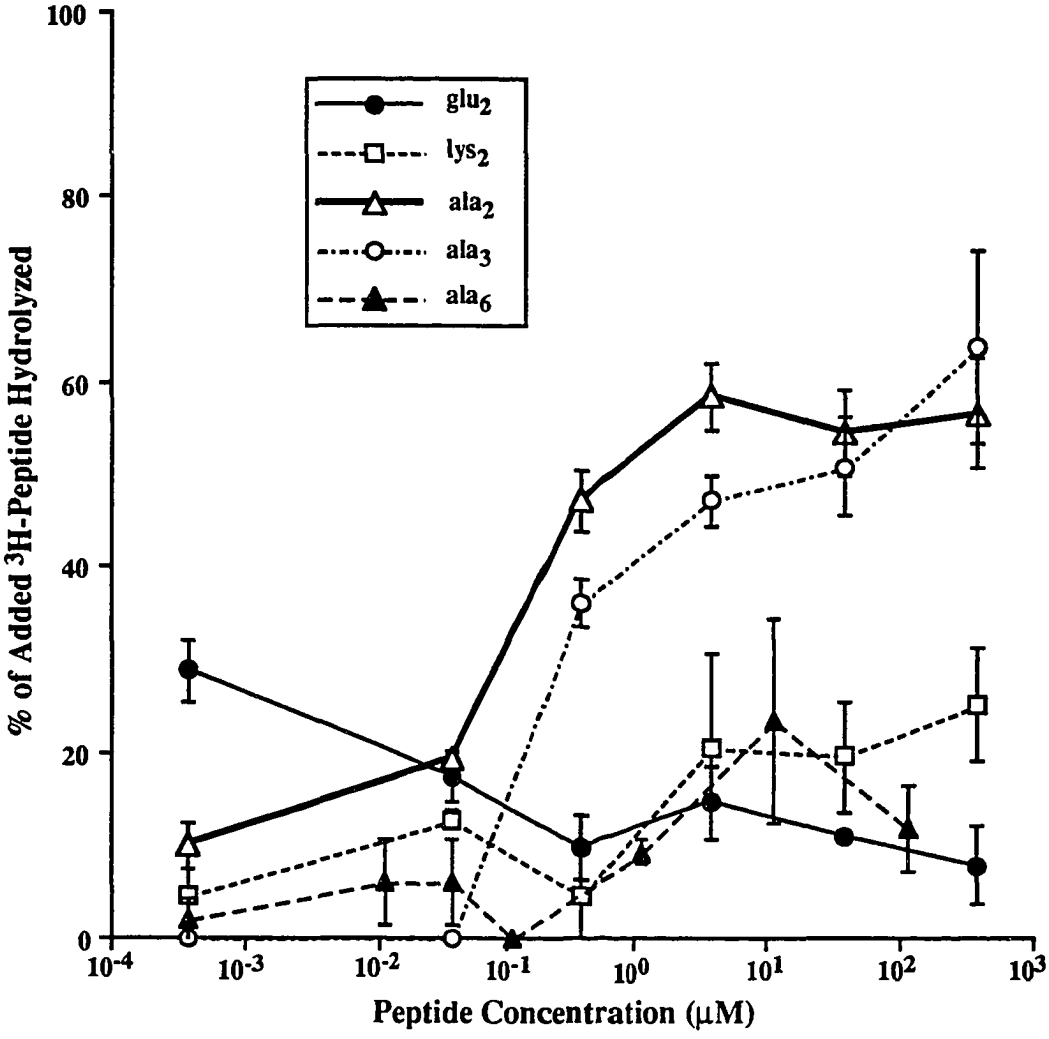


Figure 3.6. Effect of Concentration on Hydrolysis of ^{3}H-Peptides in Resurrection Bay Sediments. Incubation time is 18 minutes.

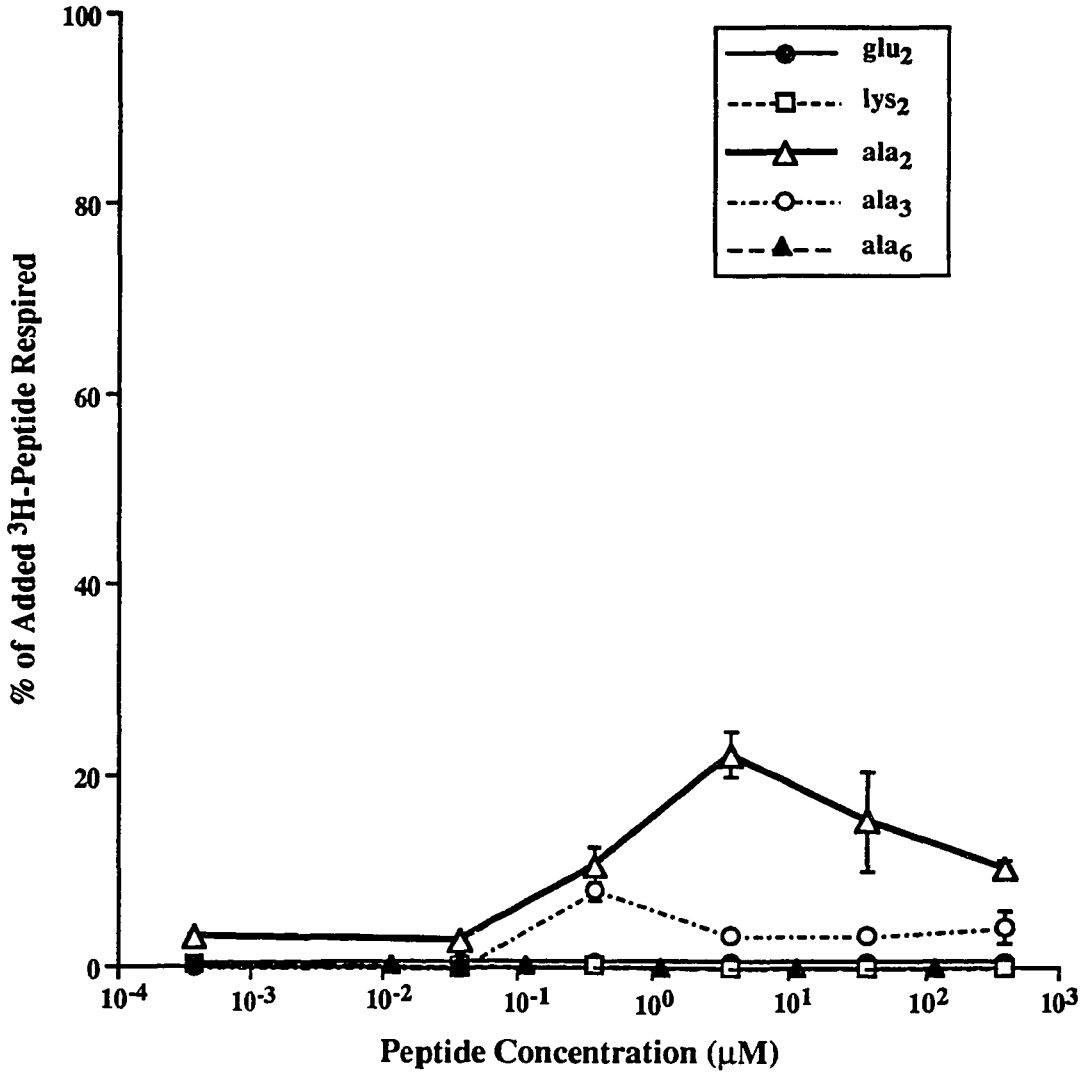


Figure 3.7. Effect of Concentration on Respiration of ^{3}H-Peptides in Resurrection Bay Sediments. Incubation time is 18 minutes.

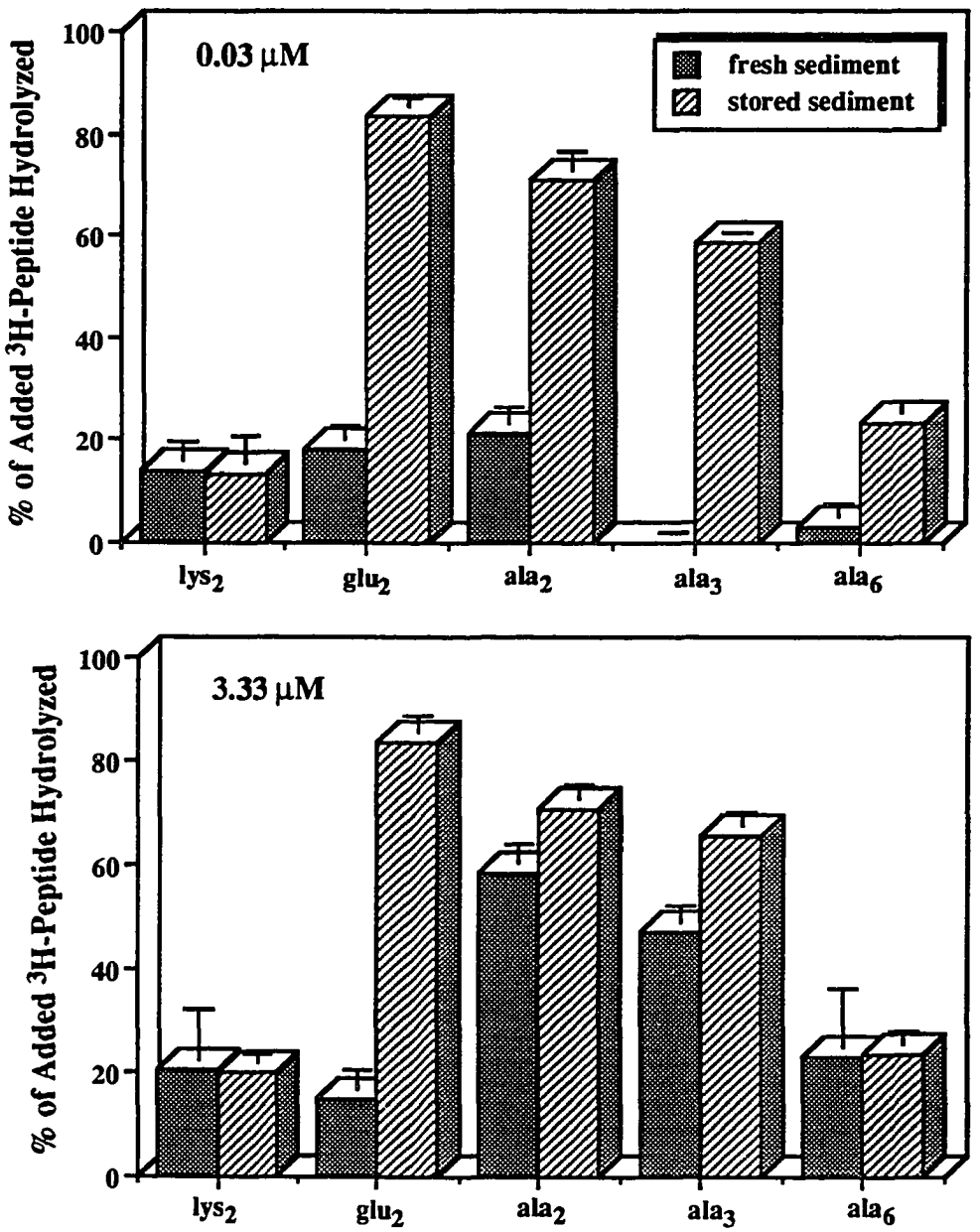


Figure 3.8. Hydrolysis of ^{3}H-Peptides in Fresh and Stored Sediments from Resurrection Bay.

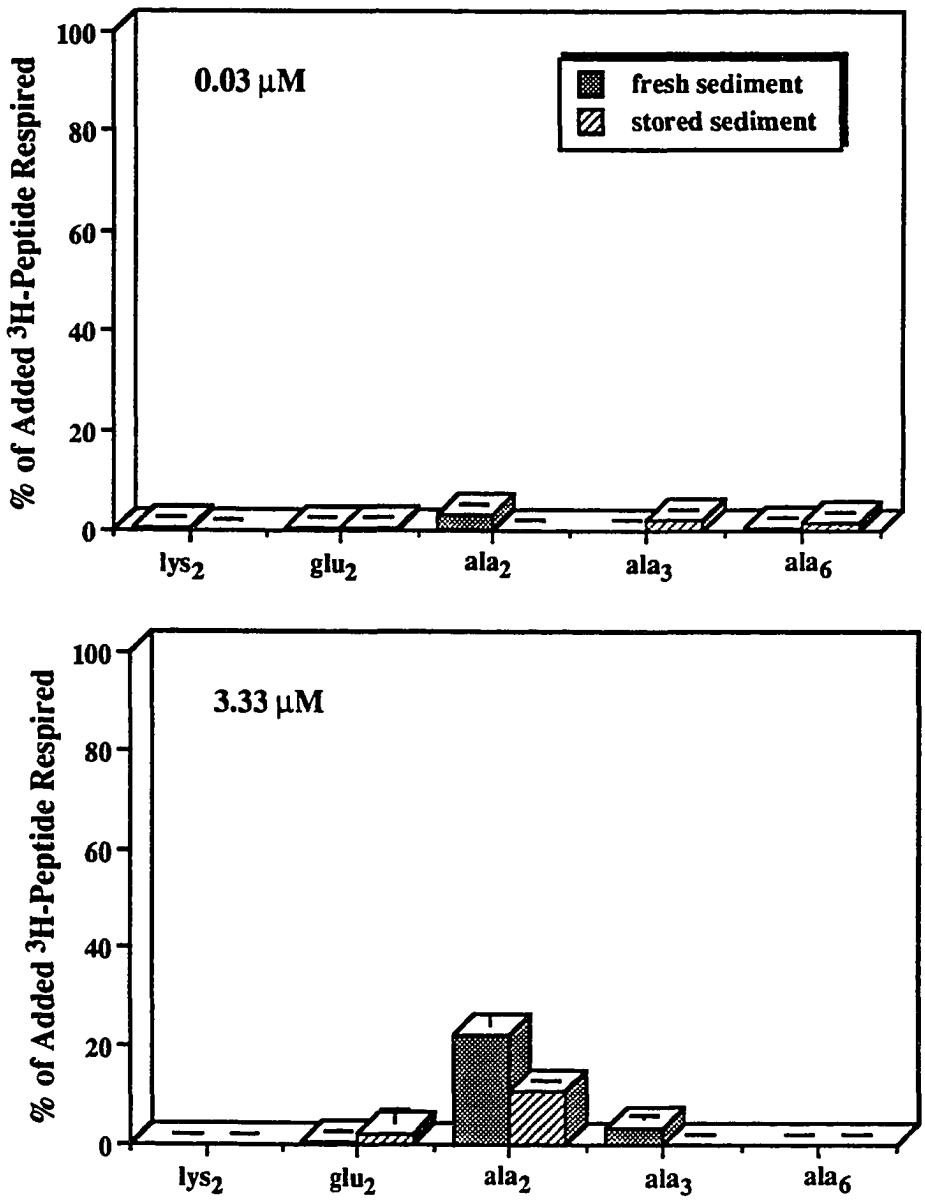


Figure 3.9. Respiration of ^{3}H-Peptides in Fresh and Stored Sediments from Resurrection Bay.

Adsorption of ^{3}H-peptides in sediments In untreated, freshly-collected sediments, adsorption of all of the peptides increased during the first hour, although the increase was not significant for ^{3}H-ala$_2$ since its adsorption was low (Figure 3.10). Considering the variability among replicates, there was no clear pattern in the change of adsorption with time between 1 and 50 hours. The adsorption of ^{3}H-peptides was in the order: lys$_2$ ≥ glu$_2$ ≥ ala$_6$ > ala$_2$.

The variation of adsorption with concentration was different for different peptides (Figure 3.11). From 0 to 333 μM, the adsorption of ^{3}H-glu$_2$ and ala$_6$ varied little in terms of the proportion of added activity adsorbed, but the adsorption of ^{3}H-lys$_2$ increased while the adsorption of ^{3}H-ala$_3$ decreased. At concentrations less than 3 μM, the adsorption of ^{3}H-ala$_2$ increased slightly with concentration, but it decreased with concentration above 3 μM. For ^{3}H-ala$_2$ and ala$_3$ at high concentrations, the decrease in the proportion adsorbed seemed to match the increase in hydrolysis (Figure 3.6). However, in all cases, adsorption in terms of the μmoles of peptide adsorbed per gram of sediment increased with concentration.

Comparing the adsorption of ^{3}H-peptides in untreated sediments and in autoclaved sediments (Figure 3.12), the adsorption in autoclaved sediments was similar (F test with $\alpha = 0.05$, Mendenhall 1987; APPENDIX III, Table A.6) to that in untreated sediments, except that the adsorption of ^{3}H-glu$_2$ in untreated sediment was greater than that in autoclaved sediment. This may have been due to the glutamic acid concentration in pore water of autoclaved sediment being 40 times greater than that of untreated sediment. This is in contrast to alanine and lysine, for which the concentration was only 4 to 10 times greater after autoclaving. The adsorption of ^{3}H-ala$_6$ and lys$_2$ in autoclaved sediments did not increase significantly for a 2 hour-incubation time (Figure 3.13). There was neither hydrolysis nor respiration in autoclaved sediments after 2 hours.

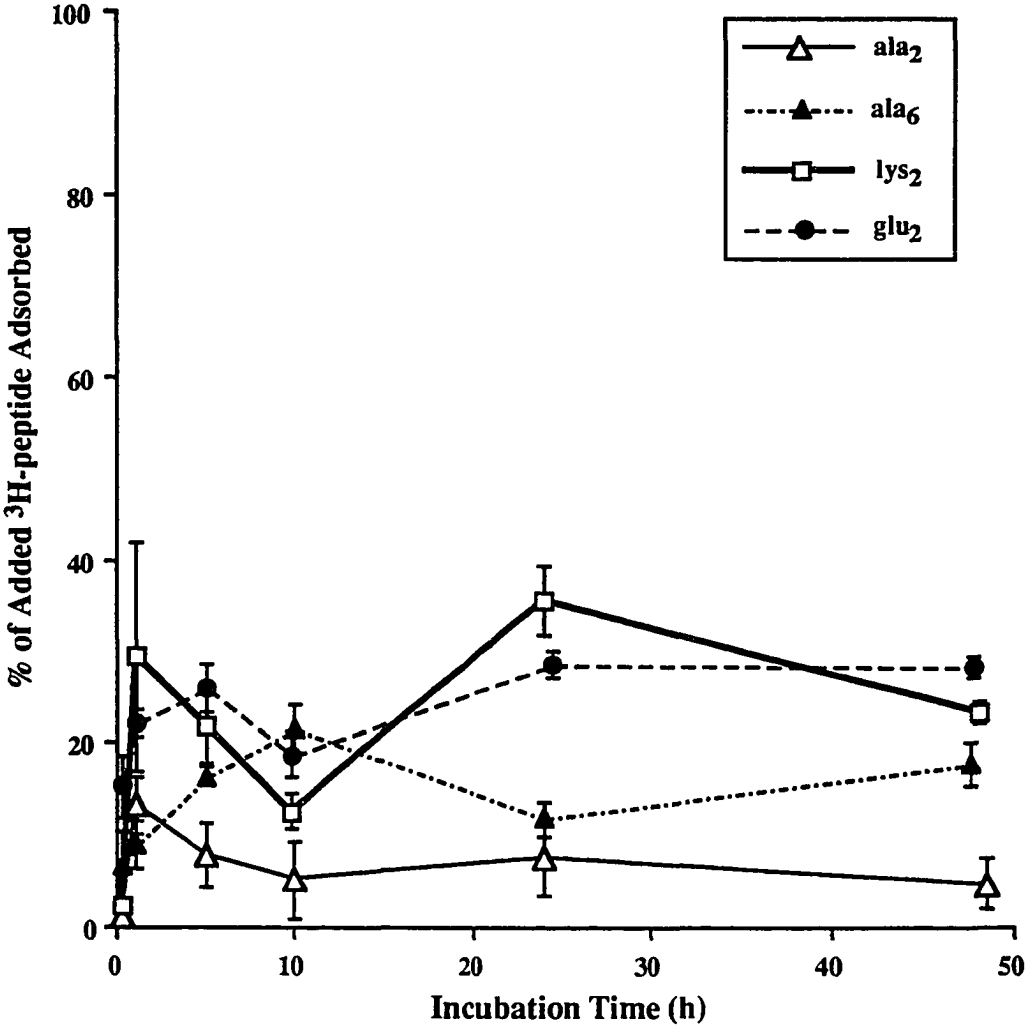


Figure 3.10. Adsorption of ^{3}H-Peptides (at 0.03 μM Initial Concentration) by Resurrection Bay Sediments.

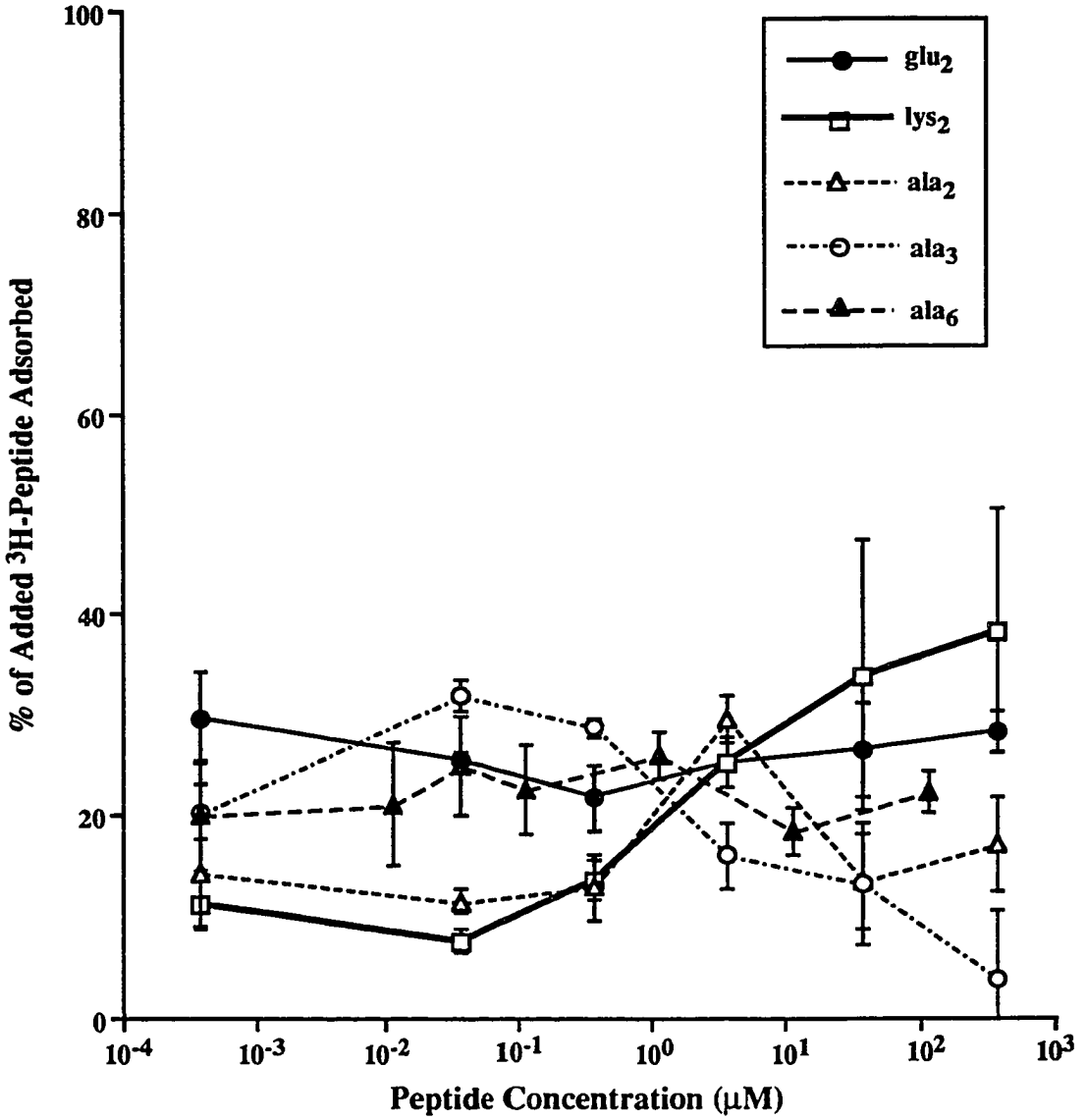


Figure 3.11. Effect of Peptide Concentration on Adsorption of ^{3}H-Peptides by Resurrection Bay Sediments.

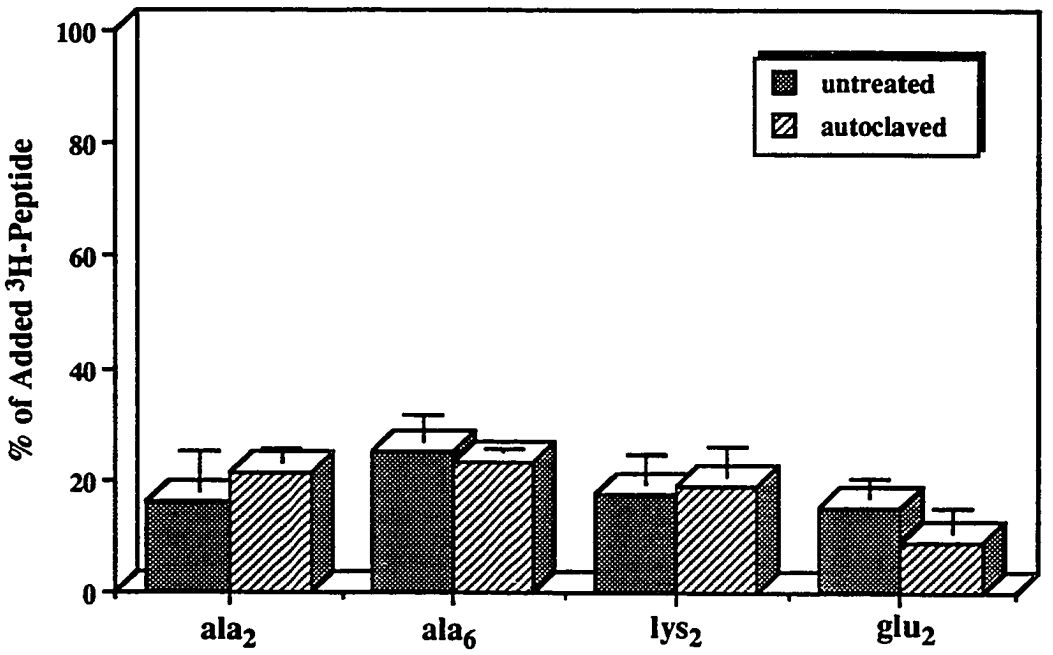


Figure 3.12. Adsorption of ^{3}H-Peptides (at 0.03 μM Initial Concentration) by Resurrection Bay Sediments.

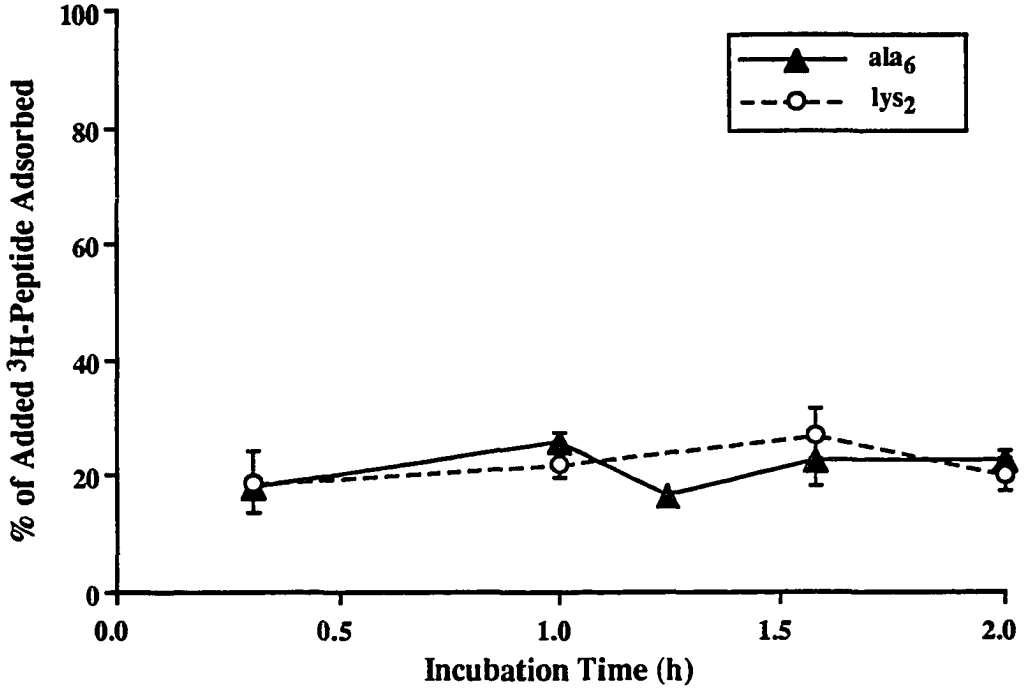


Figure 3.13. Adsorption of ^{3}H-Peptides (at 0.03 μM Initial Concentration) by Autoclaved Sediments from Resurrection Bay.

Extraction experiments (Sodium) citrate solution did not exchange adsorbed ^{3}H-peptides from sediments, and CsCl solution removed less than 10% (Figure 3.14). HCl solution extracted more adsorbed peptides than did NaOH. At 0.03 μM concentration, 100% of ^{3}H-glu$_2$ and lys$_2$, 90% of ^{3}H-ala$_2$, and 65% of ^{3}H-ala$_6$ were extracted by the HCl solution, while NaOH extracted 80 to 90% of the adsorbed ^{3}H-glu$_2$ and lys$_2$, 10% of ^{3}H-ala$_2$, and 30% of ^{3}H-ala$_6$. The extraction of peptides adsorbed at different concentrations (0.03 μM and 3.33 μM) was similar.

HCl extraction of ^{3}H-peptides adsorbed to untreated sediments decreased with incubation time (Figure 3.15). The extraction of adsorbed ^{3}H-ala$_2$ was low (less than 20%) at each time point, which was related to the low adsorption (Figure 3.10). The extraction of ^{3}H-ala$_6$ decreased with time, except that at 48 hours the extraction was unexpectedly high. All of the adsorbed ^{3}H-lys$_2$ was extracted by HCl until 24 hours, when the fraction decreased to 50%. The extraction of adsorbed ^{3}H-glu$_2$ decreased monotonically from 90% at 15 minutes to 10% at 48 hours. For ^{3}H-ala$_2$ and ala$_6$, recovery (the sum of all fractions) of added ^{3}H-activity became only slightly less than 100% with time. However, for ^{3}H-glu$_2$ and lys$_2$, the recovery was much lower after 5 hours (APPENDIX IV, Figure A.6).

In the concentration range from 0 to 333 μM (100 μM for ^{3}H-ala$_6$), the extraction of the adsorbed ^{3}H-glu$_2$, lys$_2$ and ala$_6$ did not change significantly with concentration: 90 to 100% was extracted (Figure 3.16). However, above 3.33 μM, the extraction of adsorbed ^{3}H-ala$_2$ decreased from 90 to 100% to 60 to 80%, while for ^{3}H-ala$_3$, the change in extraction with concentration was reversed. Since adsorption of peptides was relatively small compared to hydrolysis, the unextractable amount of adsorbed ^{3}H-peptide activity did not greatly affect the total ^{3}H-recovery. The total recovery of added ^{3}H-activity did not change significantly with concentration; 90 to

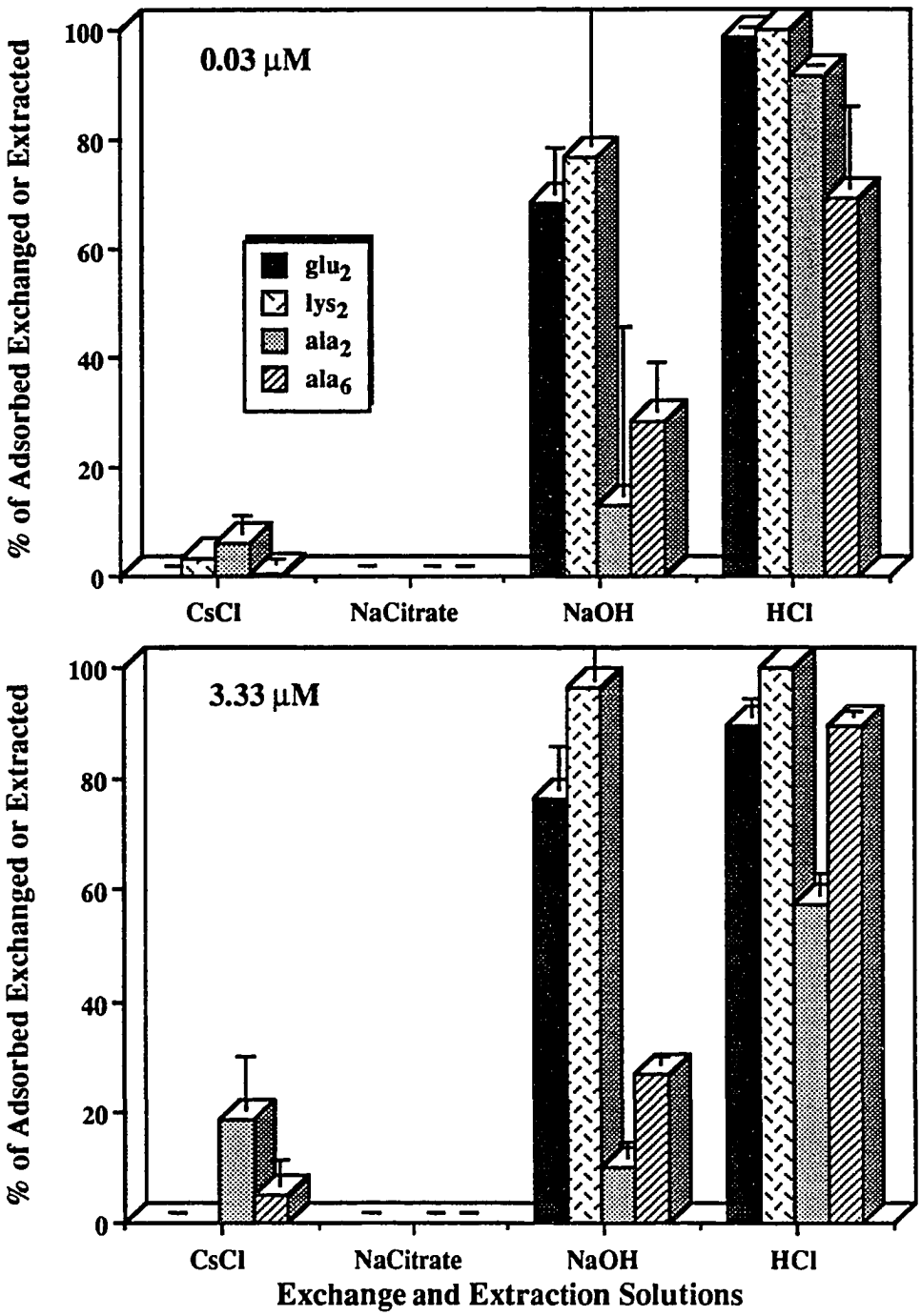


Figure 3.14. Exchange and Extraction of Adsorbed ^{3}H-Peptides from Resurrection Bay Sediments.

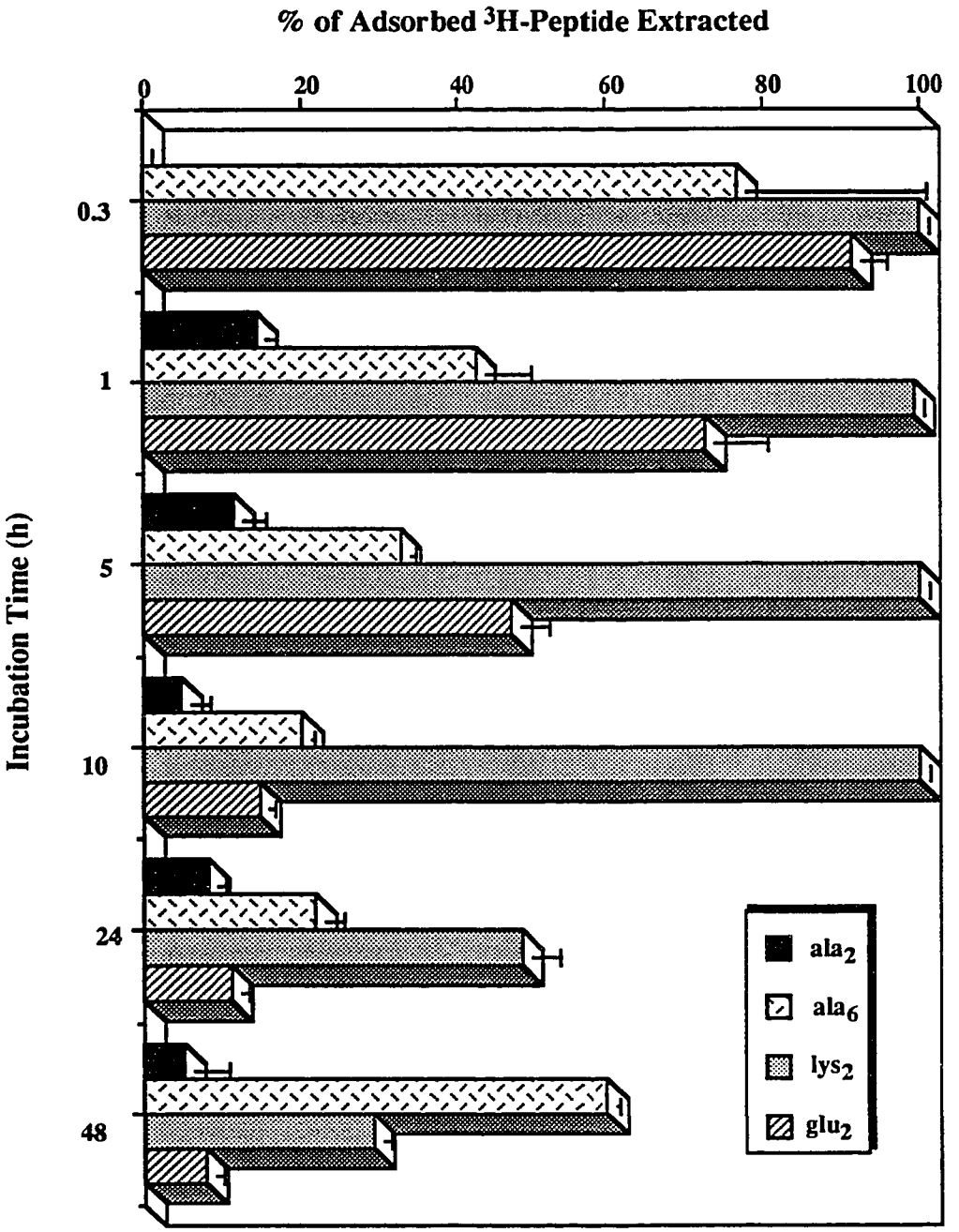


Figure 3.15. Acid Extraction of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) from Resurrection Bay Sediments.

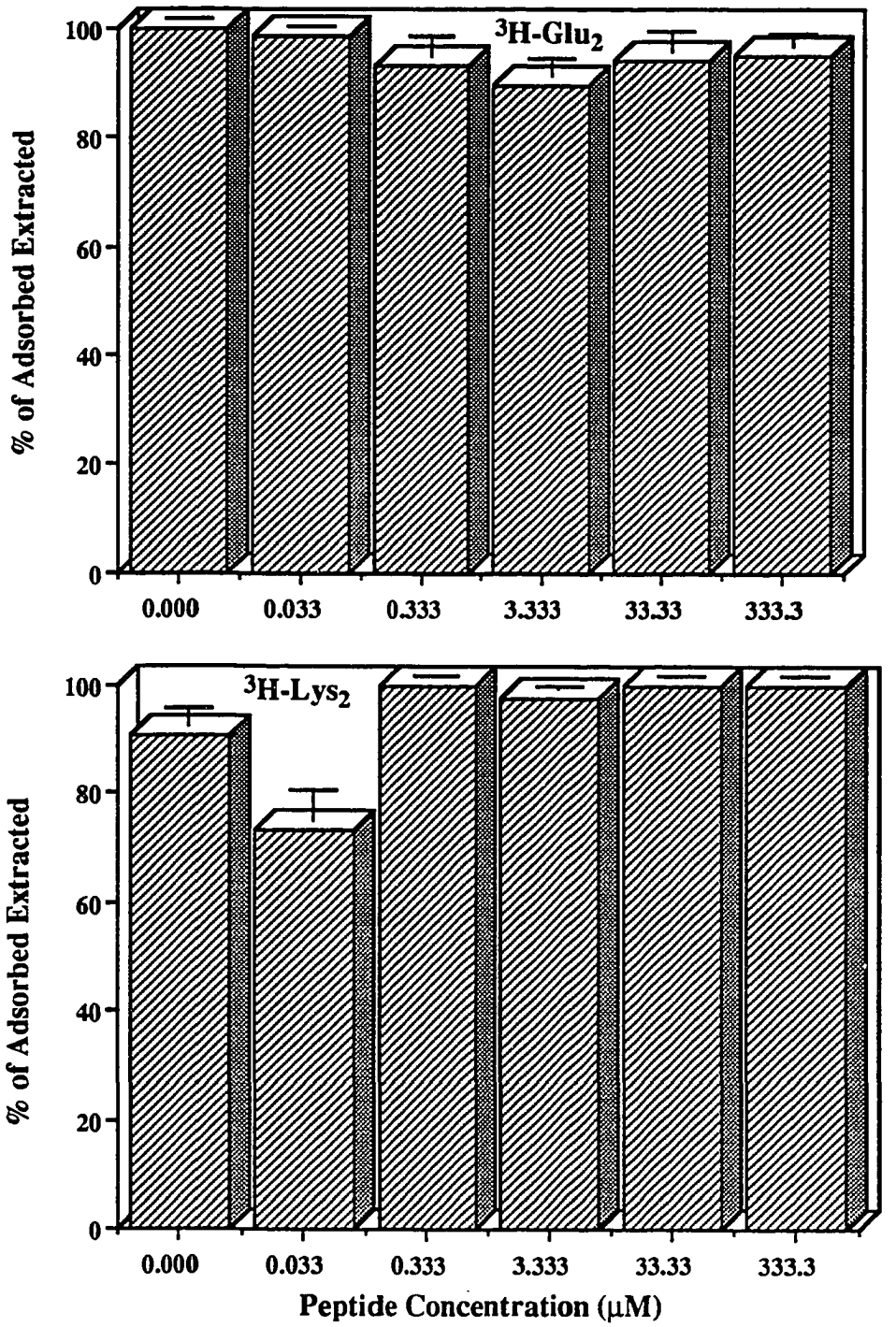


Figure 3.16. Acid (0.3 N HCl) Extraction of Adsorbed ^{3}H-Peptides from Resurrection Bay Sediments.

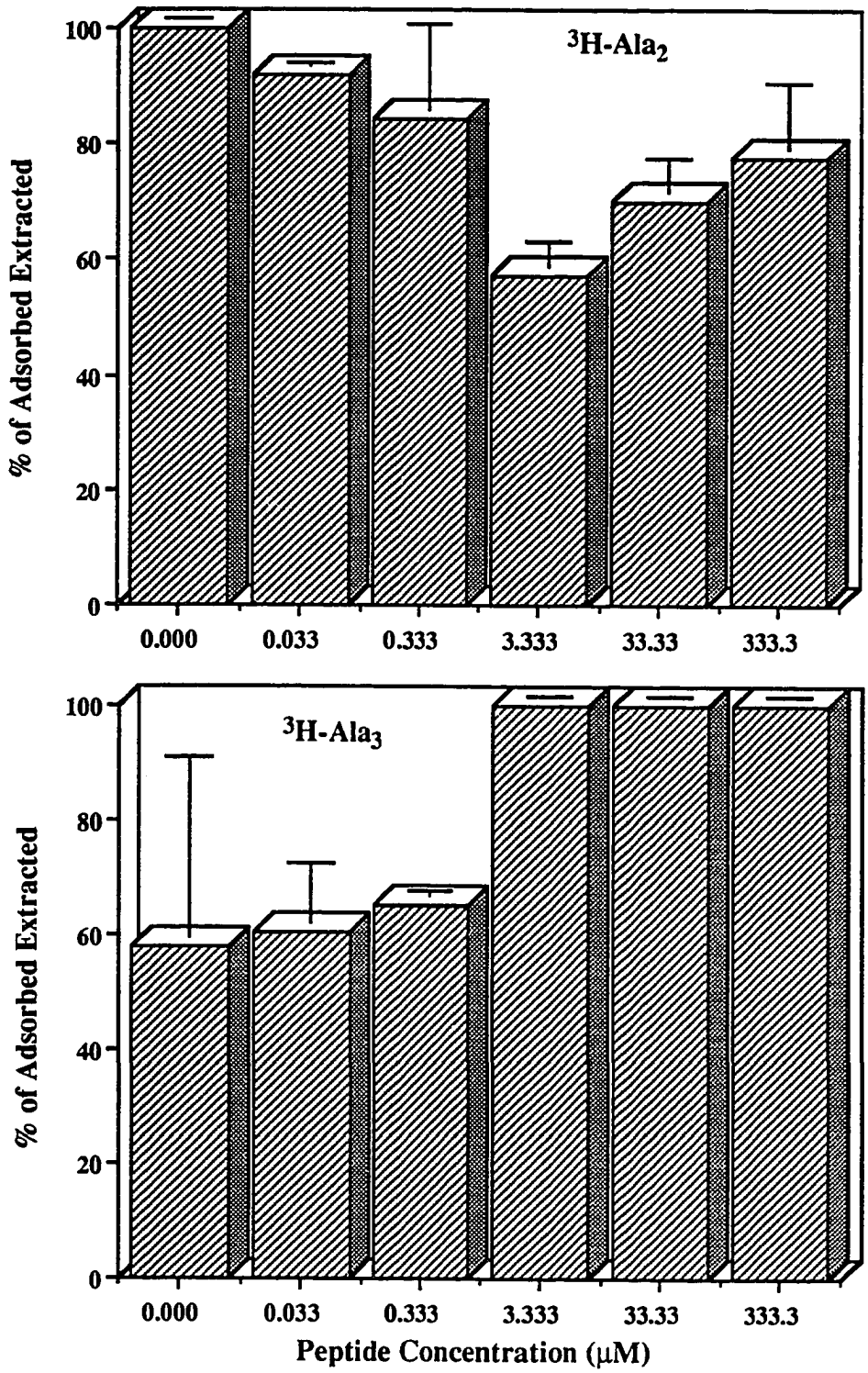


Figure 3.16. (continued)

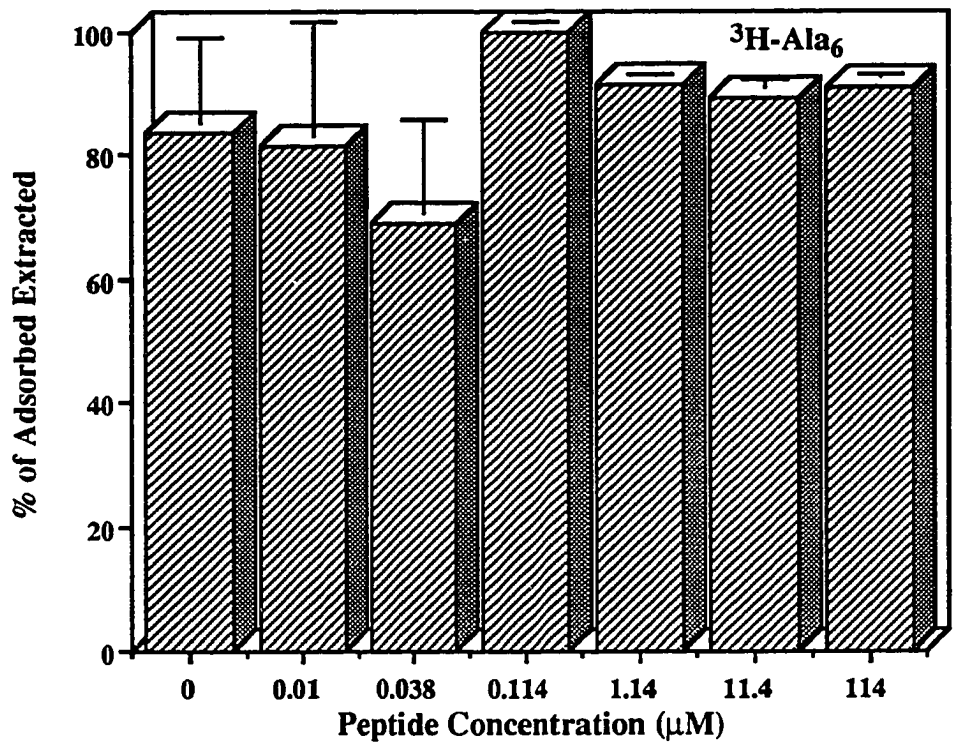


Figure 3.16. (continued)

100% of the added ^{3}H-activity was recovered at each concentration (APPENDIX IV, Figure A.7).

The extraction of ^{3}H-peptides adsorbed in untreated sediments was 2 to 3 times greater than that in autoclaved sediments, except it was similar for ^{3}H-glu$_2$ (Figure 3.17). In both untreated and autoclaved sediments, the extraction of adsorbed ^{3}H-ala$_2$ and glu$_2$ was greater than that of ^{3}H-ala$_6$ and lys$_2$.

Pretreatment experiments The adsorption of different ^{3}H-peptides was affected differently by the pretreatment of sediments. In Figure 3.18, the adsorption in untreated sediments is compared with that in pretreated sediments. The adsorption of ^{3}H-glu$_2$ and lys$_2$ in sediments that were rinsed with seawater was only half of that in untreated sediments while the adsorption of ^{3}H-ala$_2$ increased 2.5 times. Seawater rinsing did not affect the adsorption of ^{3}H-ala$_6$ significantly. The effect of CsCl pretreatment on the adsorption of ^{3}H-glu$_2$ and lys$_2$ was similar to that of the seawater rinse. The adsorption of ^{3}H-ala$_6$ in CsCl pretreated sediments was 60% of that in seawater-rinsed or untreated sediments. However, the adsorption of ^{3}H-ala$_2$ in CsCl pretreated sediments was 1.5 times that in untreated sediments. The adsorption of all ^{3}H-peptides was enhanced by the sodium citrate pretreatment (Figure 3.18). The adsorption of ^{3}H-glu$_2$ and ala$_2$ increased 2 times, while that of ^{3}H-lys$_2$ and ala$_6$ increased 25% and 17%.

Pretreatment of sediments affected not only the adsorption but also the acid extractability of the adsorbed peptides. No ^{3}H-lys$_2$ adsorbed to any of the pretreated sediments could be extracted. Less adsorbed ^{3}H-ala$_2$, ala$_6$ and glu$_2$ were extracted than in untreated sediments, except that the extraction of adsorbed ^{3}H-glu$_2$ in CsCl pretreated and untreated sediments was the same (Figure 3.19). More ^{3}H-peptides (except for ^{3}H-lys$_2$) adsorbed to CsCl pretreated sediments were extracted than those adsorbed to other

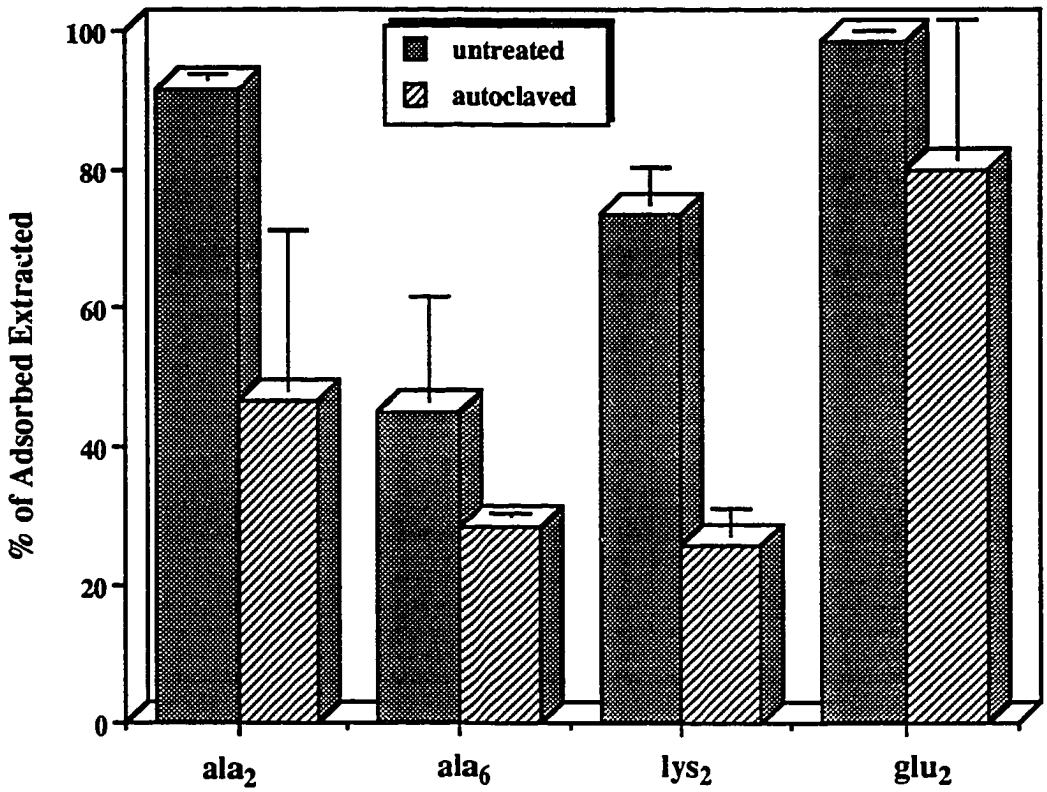


Figure 3.17. Acid Extraction of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) from Resurrection Bay Sediments.

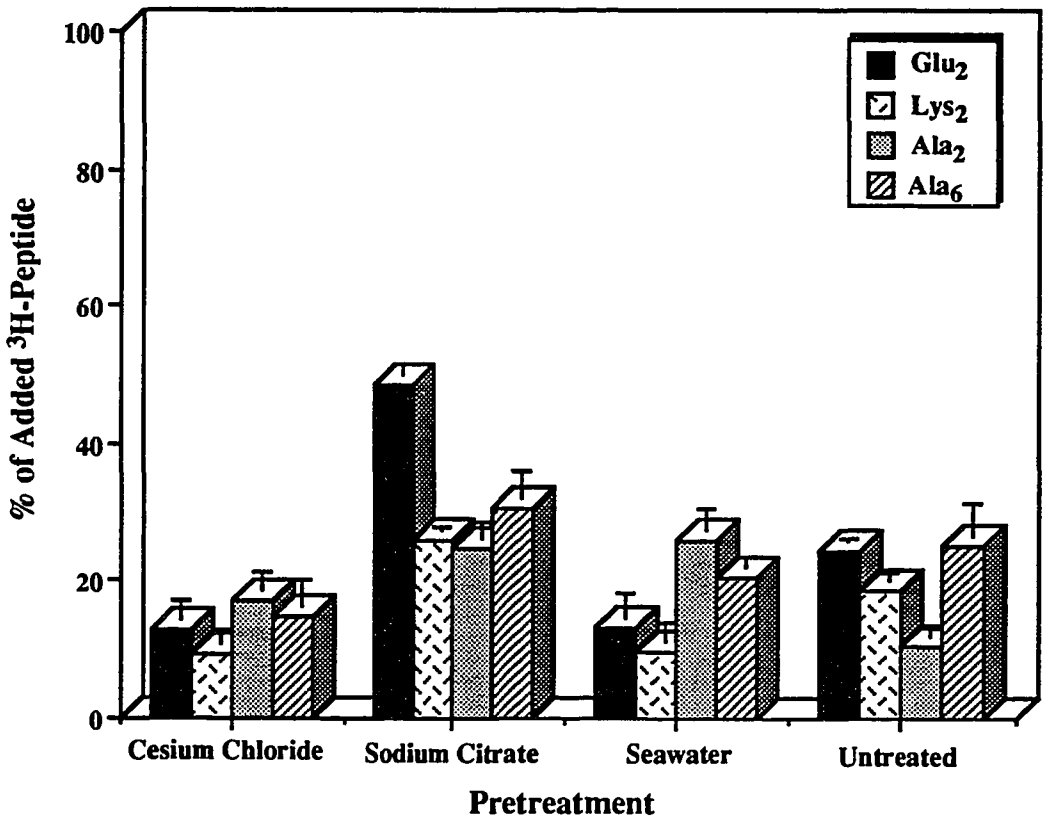


Figure 3.18. Adsorption of ^{3}H-Peptide (at 0.03 μM Initial Concentration) by Pretreated Sediments from Resurrection Bay.

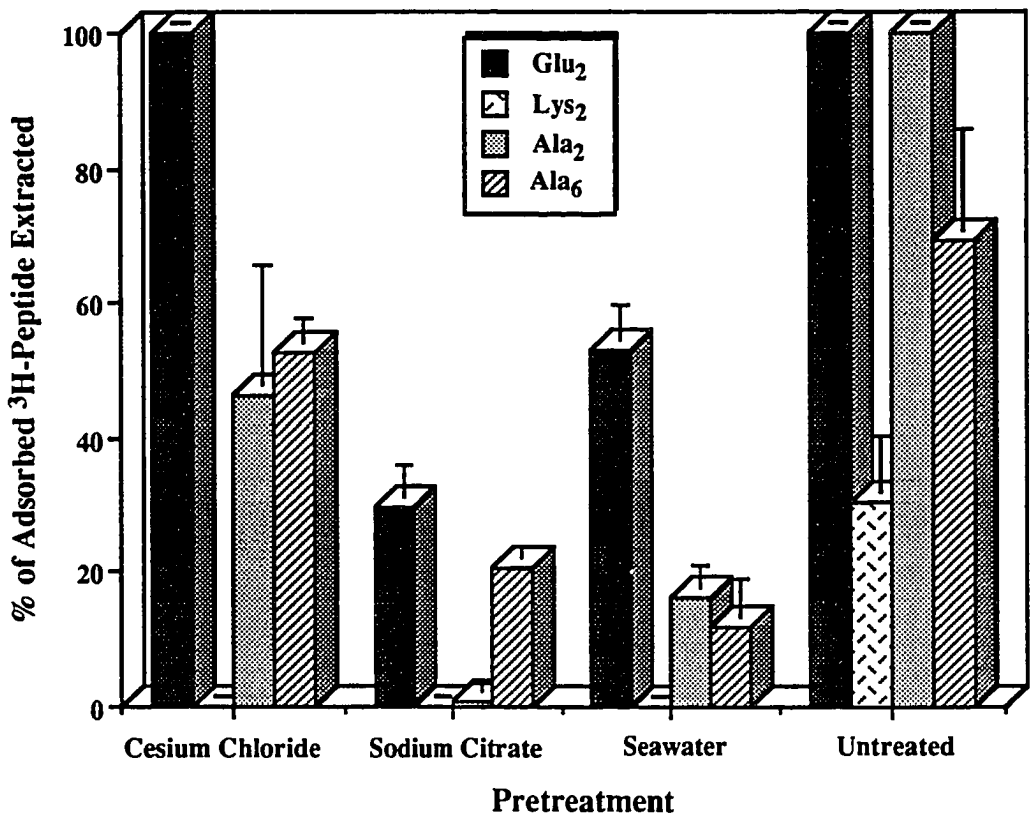


Figure 3.19. Acid Extraction of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) by Pretreated Sediments from Resurrection Bay.

pretreated sediments. The extraction of ^{3}H-glu$_2$ and ala$_6$ adsorbed after sodium citrate treatment was about 3 times lower than in untreated sediments, and no adsorbed ^{3}H-lys$_2$ and ala$_2$ were extracted. This was probably due to the neutralization of the acid by sodium citrate.

Decomposition of adsorbed ^{3}H-peptides In the decomposition experiment using pre-adsorbed peptides, if the bacteria introduced in the added, untreated sediments were only able to decompose dissolved ^{3}H-peptides (remaining in the pore water after rinsing) but not the adsorbed ones, and if no adsorbed ^{3}H-peptide was desorbed into pore water due to mixing with untreated sediment, the total ^{3}H-activity in pore water would not increase. However, after mixing, the total recovery of ^{3}H-activity in pore water increased slightly with time (Figure 3.20). The hydrolytic products of ^{3}H-peptides increased, while the dissolved ^{3}H-peptides did not decrease with time, except for ^{3}H-lys$_2$ which decreased 10 to 20%. This indicates that the some of the adsorbed ^{3}H-peptides were desorbed or hydrolyzed.

Figure 3.21 shows the hydrolysis of the adsorbed ^{3}H-peptides. The hydrolysis of ^{3}H-lys$_2$ increased from about 15% at 15 minutes to 30% after 50 hours. Less than 15% of the adsorbed ^{3}H-ala$_6$ was hydrolyzed after 50 hours. The respiration of the adsorbed ^{3}H-lys$_2$ did not increase significantly with time, and less than 5% was respired after 50 hours. Almost all of the ^{3}H-alanine hydrolyzed from ^{3}H-ala$_6$ was respired after 24 hours (Figure 3.22).

Figure 3.23 shows the change with time in dissolved ^{3}H-peptides and their decomposition products in pore water and acid extracts. During a 48 hour-incubation, ^{3}H-lysine from ^{3}H-lys$_2$ was nearly zero while the ^{3}H-alanine from alanyl peptides increased slightly with time. This may be due to a low hydrolysis rate or high adsorption of FAA. There was not much adsorbed ^{3}H-lys$_2$ extracted in HCl solution, less than 12%

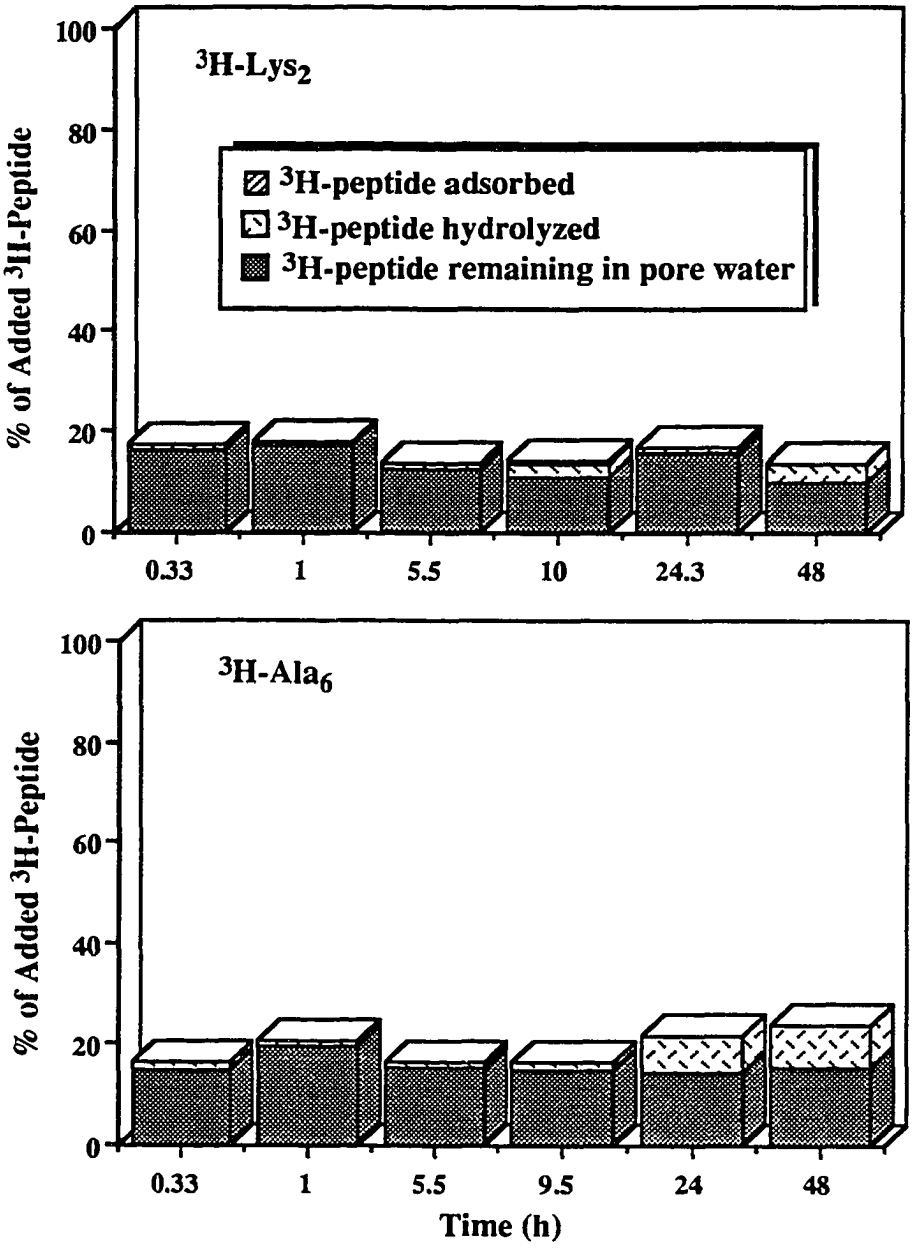


Figure 3.20. ^{3}H-Peptides (at 0.01 μM Initial Concentration) in Resurrection Bay Sediments (Autoclaved + Fresh).

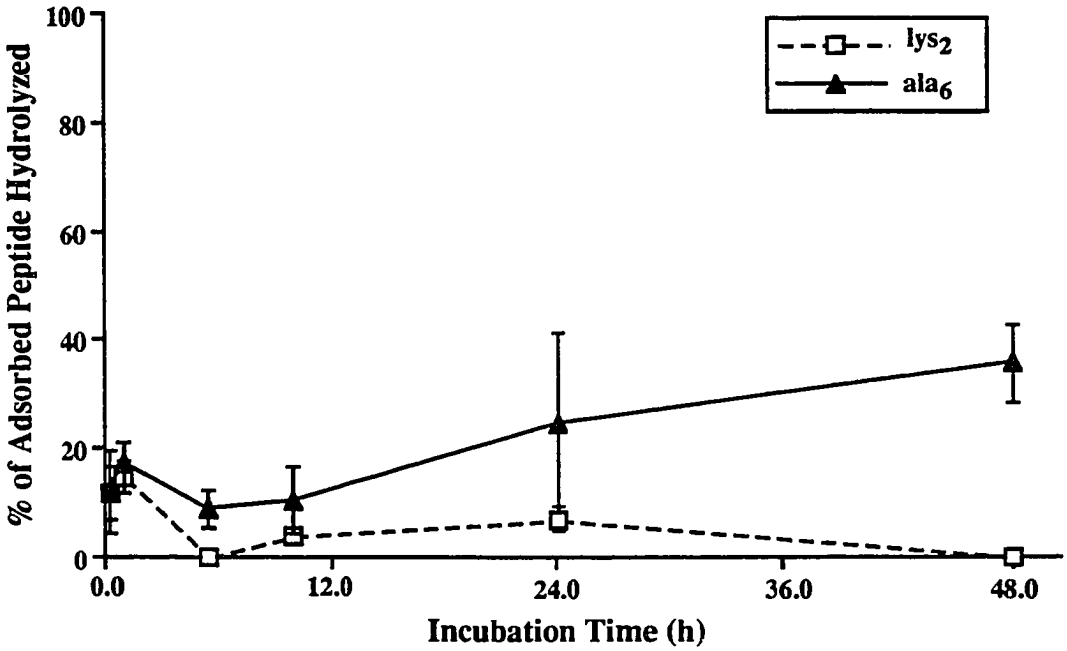


Figure 3.21. Hydrolysis of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Resurrection Bay Sediments.

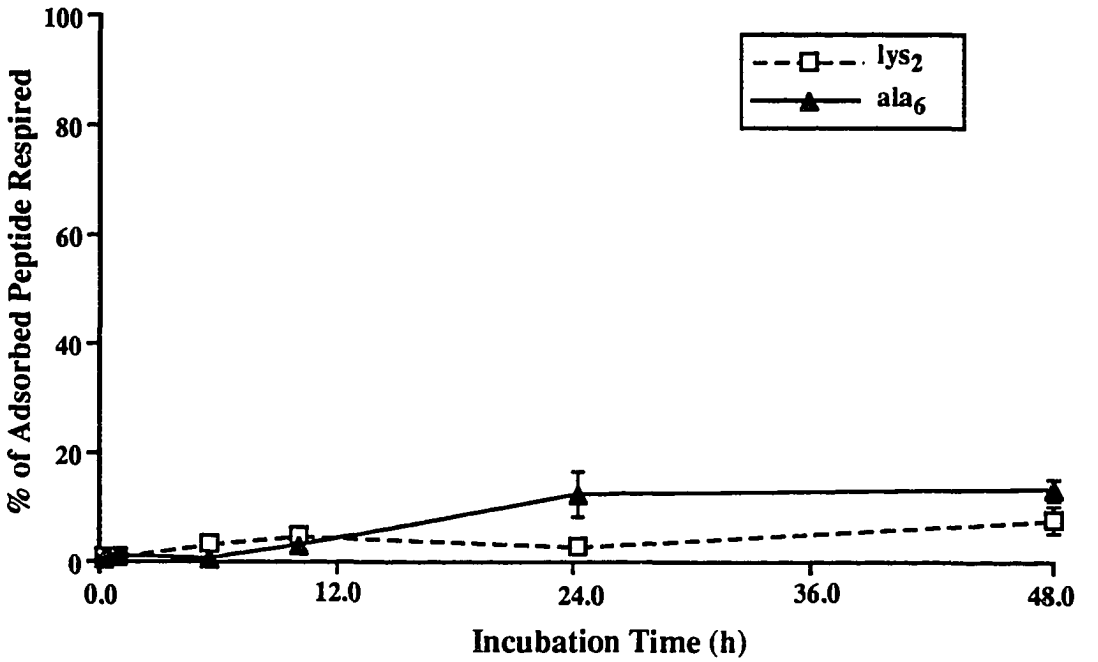


Figure 3.22. Respiration of Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Resurrection Bay Sediments.

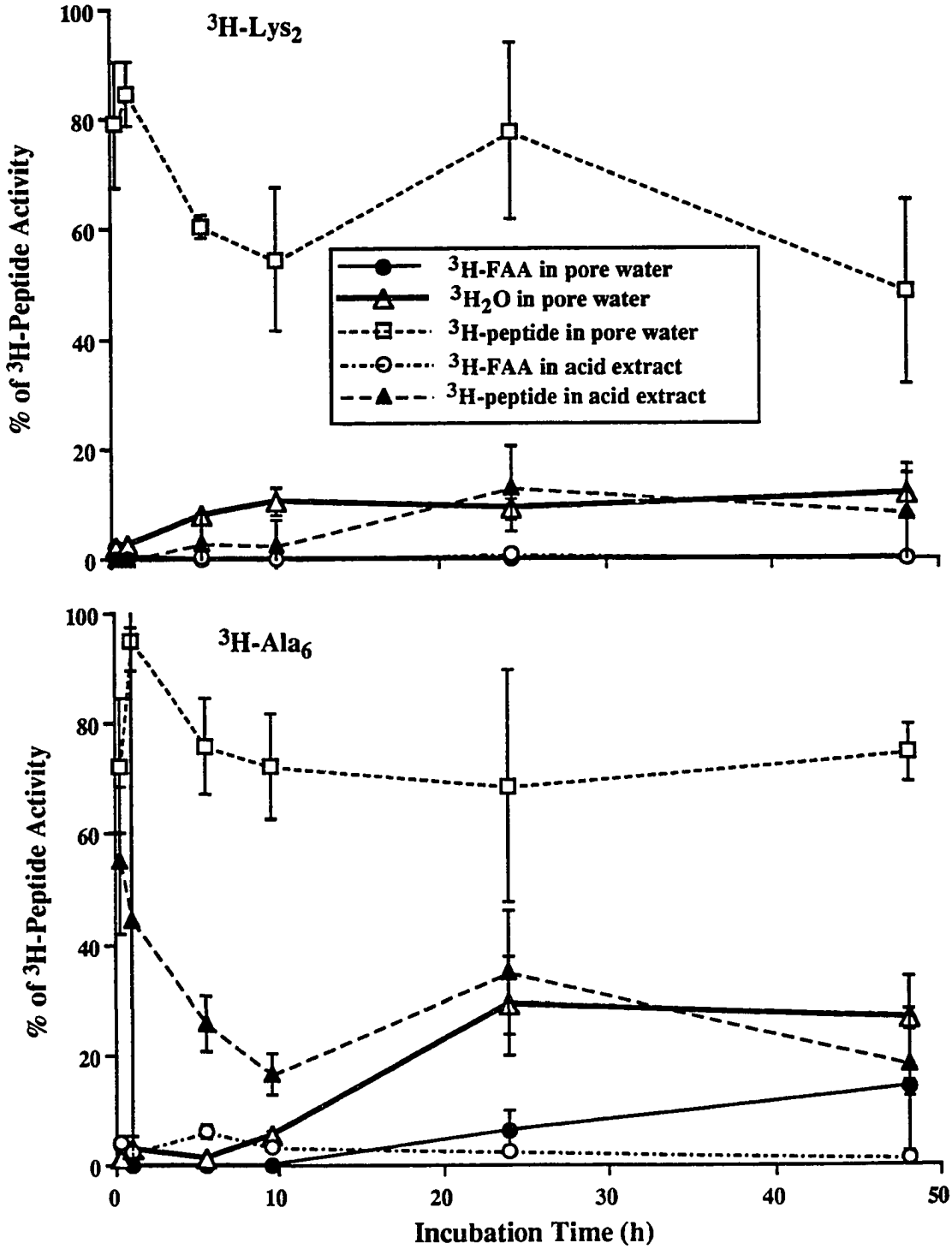


Figure 3.23. Decomposition of ^{3}H-Peptides (at 0.01 μM Initial Concentration) in Resurrection Bay Sediments (Autoclaved + Fresh).

after 48 hours. About 55% of the adsorbed ^{3}H-ala$_6$ was extracted at 15 minutes, but after 1 hour, the extracted activity decreased steadily to about 15% after 10 hours. Comparing the results in mixed autoclaved and fresh sediment with those in fresh sediment (Figure 3.5), the patterns of variation were similar, except that much more ^{3}H-lys$_2$ and ala$_6$ remained in solution and were extracted by acid in the mixed sediment. Moreover, little of the adsorbed peptides could be extracted by 0.2 N HCl, and the total recovery in hydrolyzed and extracted fractions was far less than 100% (Figure 3.24).

Discussion

Decomposition of peptides In Resurrection Bay sediments, all of the hydrolytic enzyme activity was associated with sediment particles or bacterial cells. No significant dissolved hydrolytic enzyme activity was present.

The changes in the concentrations of ^{3}H-peptides and their decomposition products in the dissolved pool over time (Figure 3.25) indicate the decomposition pathway of ^{3}H-peptides in sediments, which I hypothesize is a two-step process: hydrolysis of the peptide releasing free amino acids to pore water, then uptake of the free amino acids by bacteria. For ^{3}H-lys$_2$ and glu$_2$, the hydrolytic products (^{3}H-FAA) accumulated in the dissolved pool and reached a maximum after 1 to 5 hours, then decreased steadily with time. The accumulation lasted longer for ^{3}H-lys$_2$ than for ^{3}H-glu$_2$. However, for ^{3}H-ala$_2$ and ala$_6$, there was no accumulation of ^{3}H-FAA in the dissolved pool, but ^3H_2O increased steadily with time after 15 minutes. Whether a free amino acid accumulated in the dissolved pool was affected by the hydrolysis rate of the peptide, and the adsorption and respiration rates of the released amino acids. For the three hydrolytic products (alanine, glutamic acid and lysine), the turnover times in units of hours in Resurrection Bay sediments calculated using tracer model (which will be

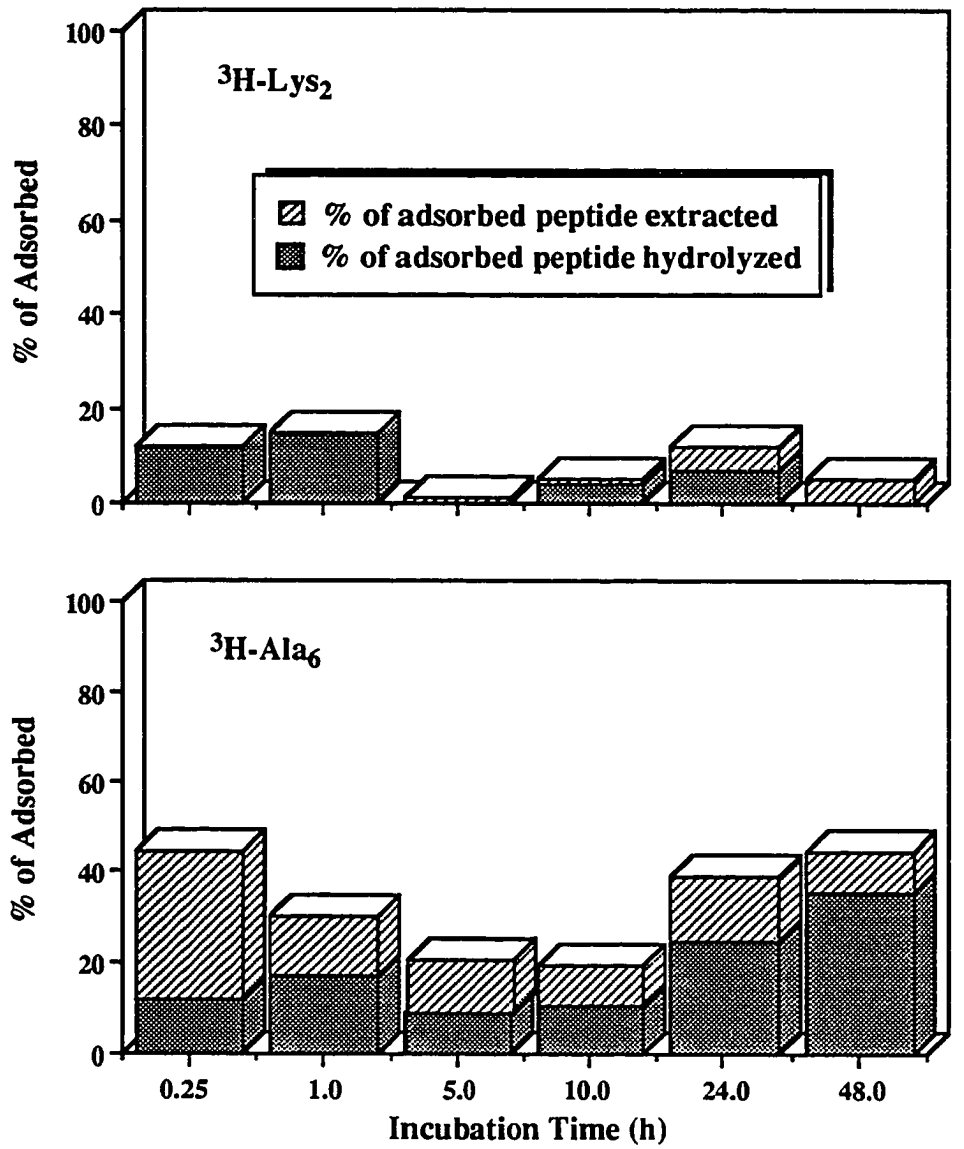


Figure 3.24. Adsorbed ^{3}H-Peptides (at 0.01 μM Initial Concentration) in Resurrection Bay Sediments (Autoclaved + Fresh).

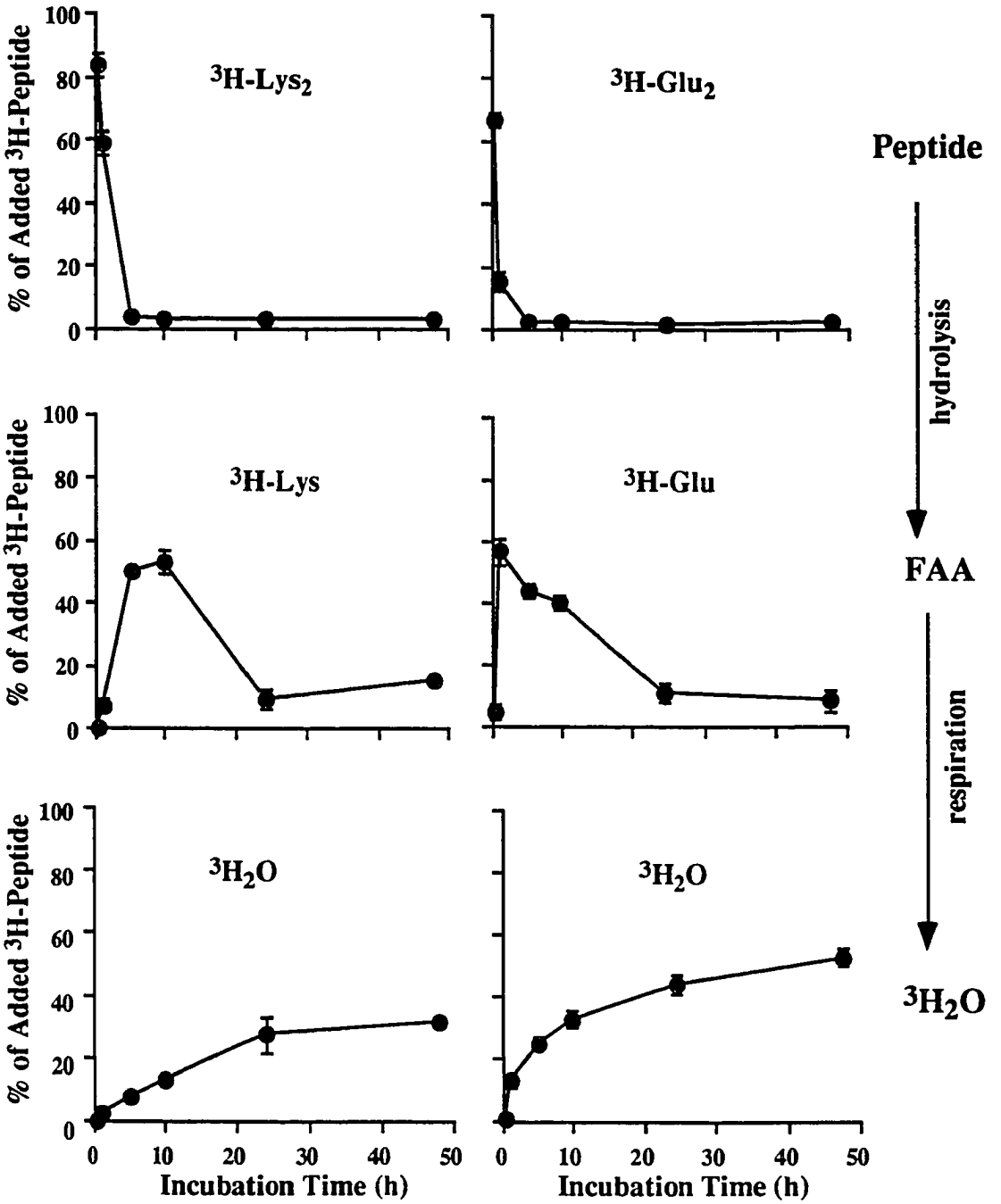


Figure 3.25. Decomposition Pathway of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Resurrection Bay Sediments.

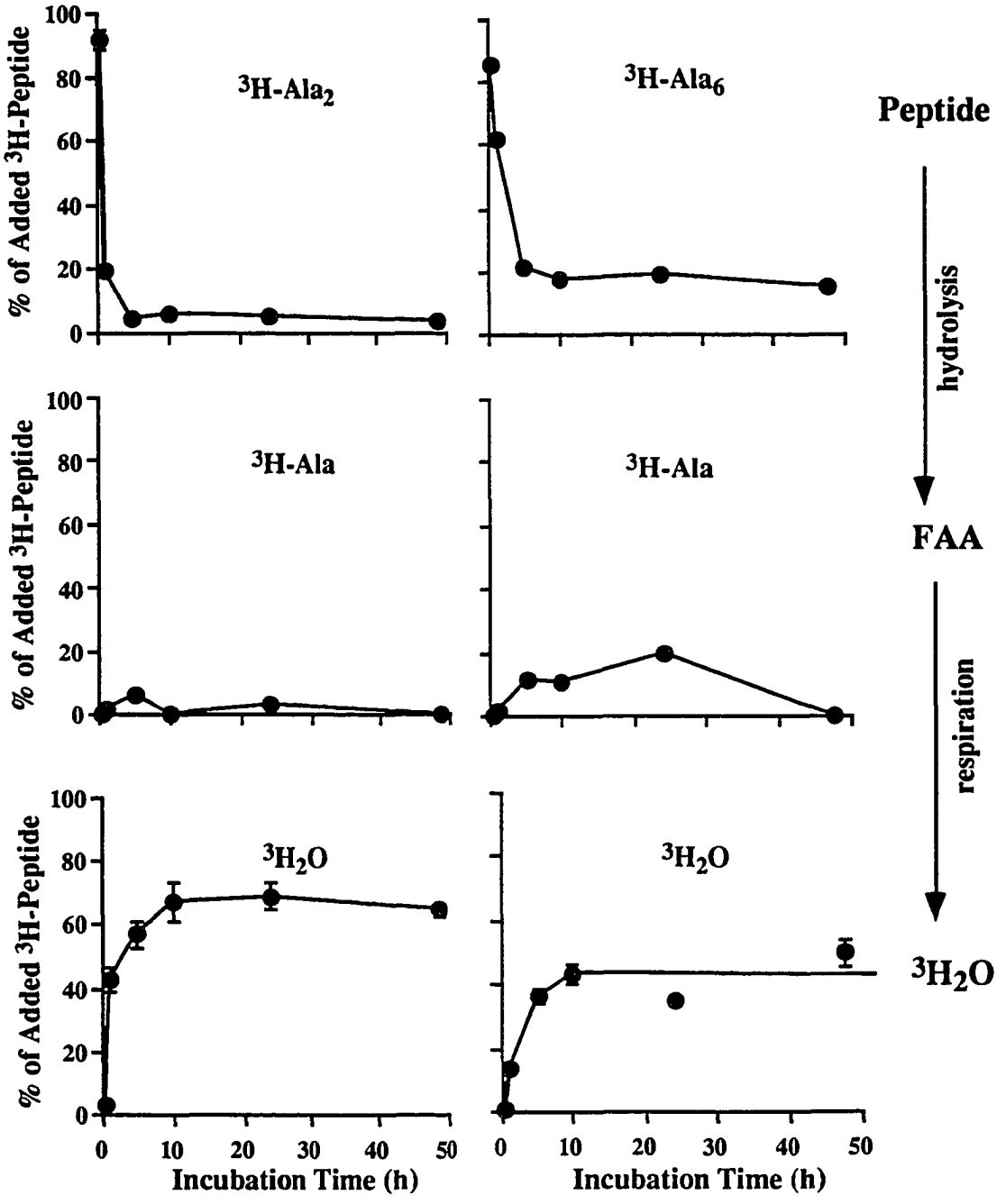


Figure 3.25. (continued)

explained in the following paragraphs) are: 0.5 - 0.8 (alanine), 0.8 (glutamic acid), 2.5 (lysine) (Table 3.1). The rapid respiration of alanine was responsible for the lack of accumulation of alanine in the dissolved pool. Also, glutamic acid and especially lysine adsorbed to a greater extent than alanine (Henrichs and Sugai 1993). Some of the lysine and glutamic acid in pore water at later times may have been desorbed from the sediment. These points will be addressed quantitatively in the discussion of the tracer model which follows.

A diffusion model and a tracer model were used to calculate the hydrolysis and respiration rates of peptides. Hydrolysis and respiration data measured at different added peptide concentrations were interpreted using the diffusion model (Wright and Burnison 1979). The plot of concentration (nmol/mL) vs. hydrolysis and respiration rates shows an approximately linear increase from 0.01 to 333 μM (Figures 3.26a, b, c, d and e). The respiration rate estimated using this model was the total rate at which peptides were respired to ^3H_2O via FAA hydrolysis. The slope of the line is the rate constant in units of mL cm^{-3} d^{-1}. The rate constants in Table 3.1 were converted to units of h^{-1} by dividing the slope by (ϕ * 24) where ϕ, equal to 0.625, is the sediment porosity. The rate constants of ^{3}H-peptide hydrolysis (k_d) were in the order: $ala_2 > ala_3 > lys_2 \geq glu_2 \geq ala_6$. The order of rate constants of overall respiration (k_{fd}) from ^{3}H-peptide to ^3H_2O was: $ala_2 > ala_3 > glu_2 \gg ala_6 > lys_2$ (Table 3.1). Although k_d values of ^{3}H-lys_2, glu_2 and ala_6 were similar, the k_{fd} value of lys_2 was 4 to 10 times smaller than those of glu_2 and ala_6, due to the much greater adsorption of lysine and lys_2.

The equations (including assumptions and simplifications) for the tracer model used to calculate peptide hydrolysis and the uptake and respiration of free amino acids were the same as those given in Chapter 2. The parameters are defined as follows:

k_1 = rate constant for bacterial uptake of ^{3}H-FAA from pore water

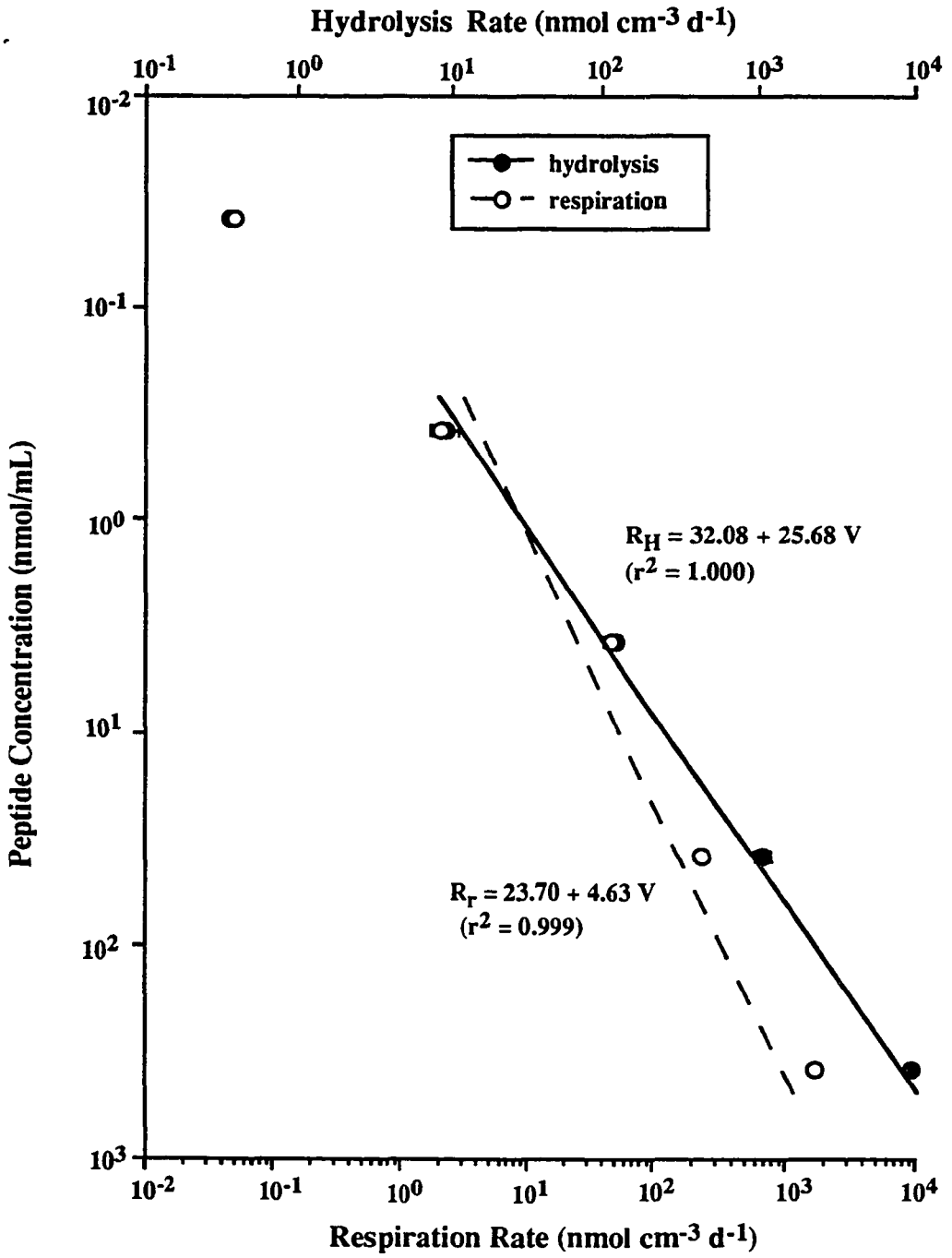


Figure 3.26a. Effect of Concentration on Decomposition of ^{3}H-Ala$_2$ in Resurrection Bay Sediments. R_H=hydrolysis rate, R_r=respiration rate, V=peptide concentration

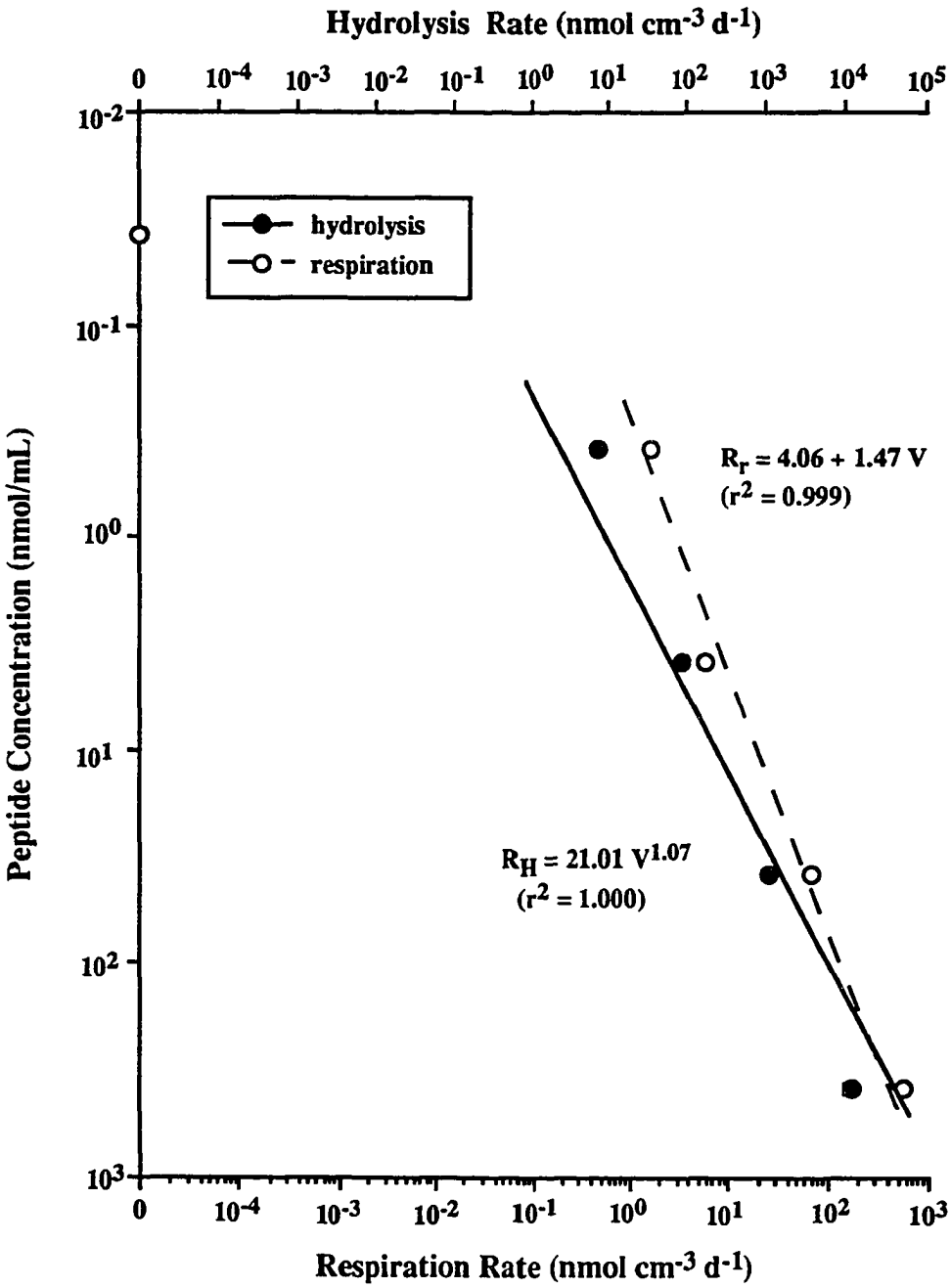


Figure 3.26b. Effect of Concentration on Decomposition of ^{3}H-Ala$_3$ in Resurrection Bay Sediments. R_H=hydrolysis rate, R_r=respiration rate, V=peptide concentration

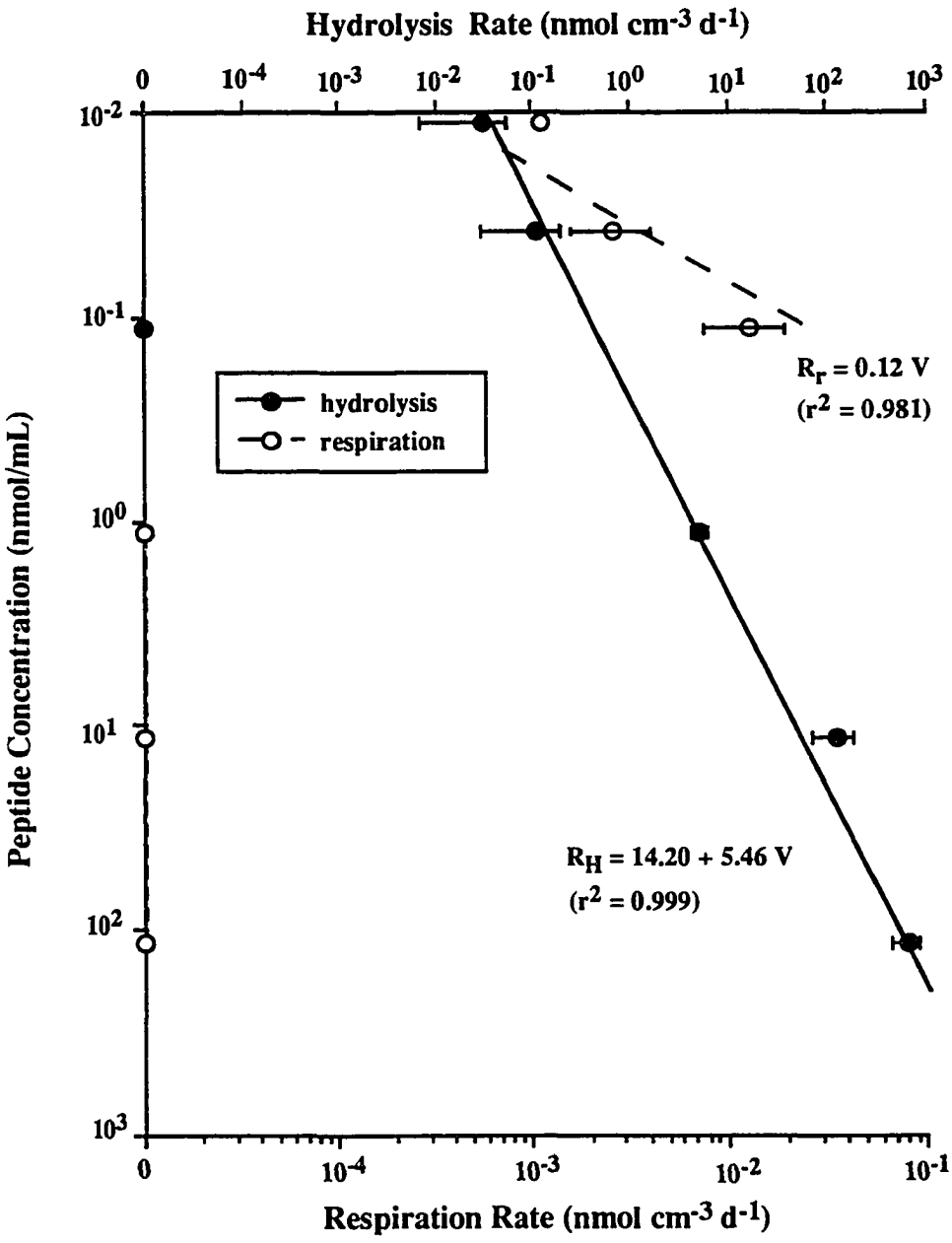


Figure 3.26c. Effect of Concentration on Decomposition of ^{3}H-Ala$_6$ in Resurrection Bay Sediments. R_H=hydrolysis rate, R_r=respiration rate, V=peptide concentration

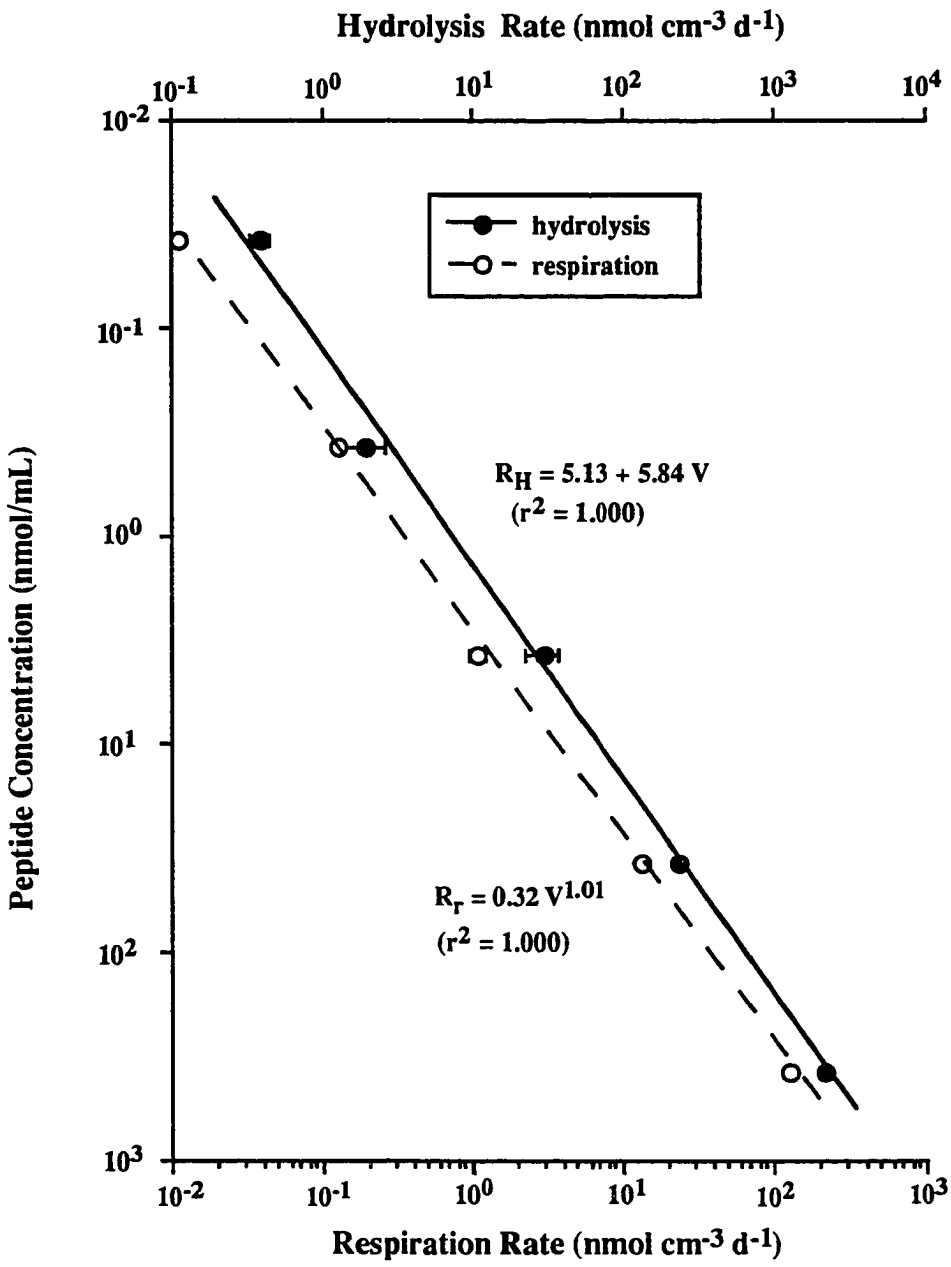


Figure 3.26d. Effect of Concentration on Decomposition of ^{3}H-Glu$_2$ in Resurrection Bay Sediments. R_H=hydrolysis rate, R_r=respiration rate, V=peptide concentration

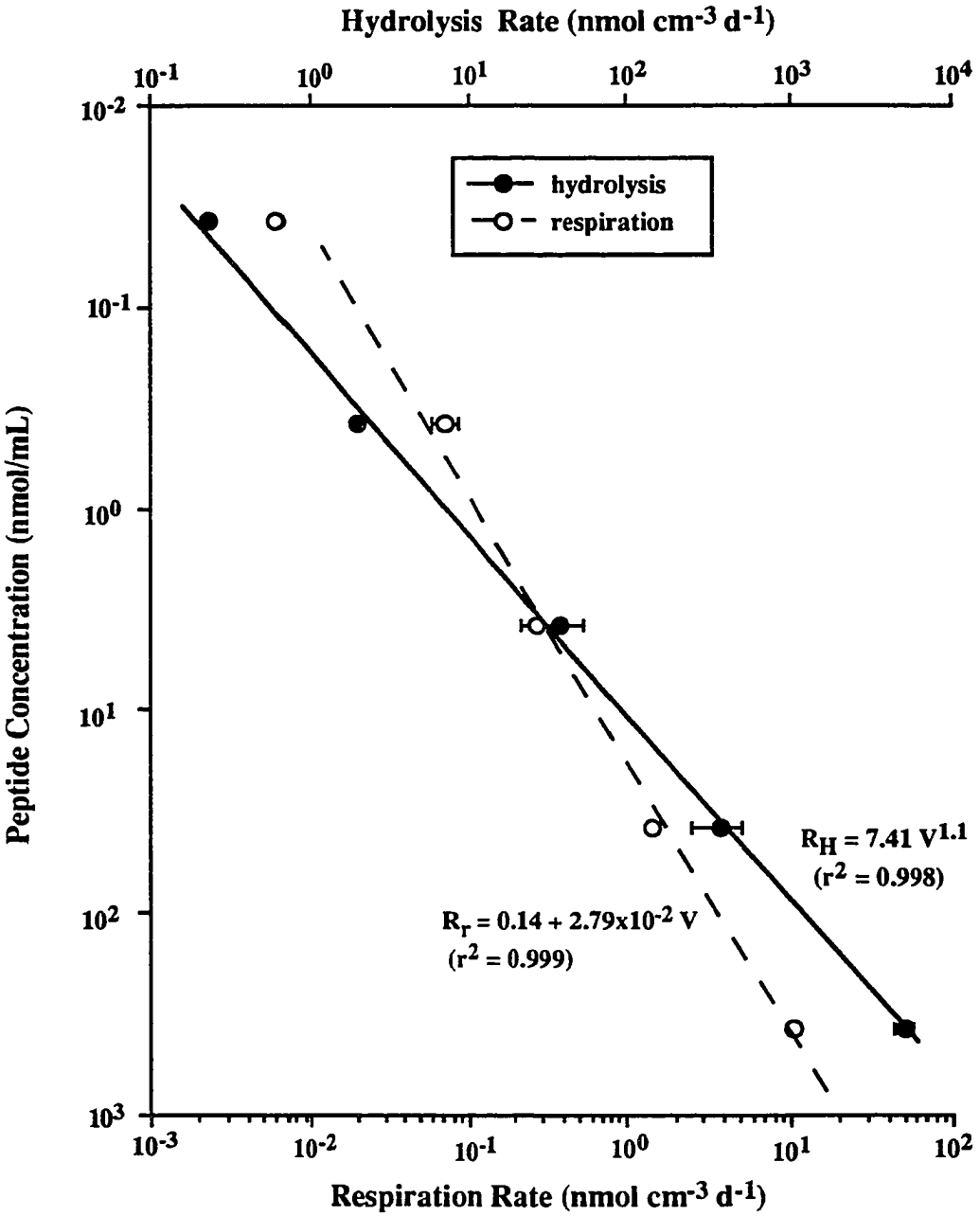


Figure 3.26e. Effect of Concentration on Decomposition of ^{3}H-Lys$_2$ in Resurrection Bay Sediments. R_H=hydrolysis rate, R_r=respiration rate, V=peptide concentration

Table 3.1. Rate Parameters of Peptide Decomposition in Resurrection Bay Sediments

^{3}H-peptide	direct measurement[1]	diffusion model[2]		tracer model[3]							
	k_{dm} (h^{-1})	k_d (h^{-1})	k_{fd}	k_1	k_2	k_3	R (h^{-1})	k_p	k_a	k_{PAE}	S
di-alanine	0.703	1.712	0.309	2.0	0.04	0.015	0.20	1.20	0.05	0.02	3.69
tri-alanine		1.400	0.098								
hexa-alanine	0.103	0.364	0.008	1.30	0.04	0.03	0.40	0.20	0.05	0.03	7.99
di-lysine	0.470	0.494	0.002	0.40	0.03	0.20	0.40	0.40	0.15	0.01	8.50
di-glutamic acid	0.600	0.389	0.021	1.20	0.008	0.25	0.40	0.60	0.05	0.04	7.53

[1] k_{dm} is the rate constant of ^{3}H-peptide hydrolysis calculated by [% of hydrolysis]/t.

[2] k_d and k_{fd} are the rate constants of ^{3}H-peptide hydrolysis and ^{3}H-FAA respiration, respectively, calculated using the model described by Wright and Burnison (1979).

[3] k_1, k_2, k_3, R, k_p, k_a and k_{PAE} are parameters defined in the text. S is the error of the model fit to the data, the square root of the sum of squared residuals divided by the number of data points, in units of % of added radioactivity.

k_2 = rate constant for bacterial uptake of ^{3}H-FAA from acid-extractable pool

k_3 = rate constant for ^{3}H-FAA adsorption plus bacterial uptake of ^{3}H-FAA from pore water into the acid-extractable pool

k_P = rate constant for ^{3}H-peptide hydrolysis in pore water

k_a = rate constant for peptide adsorption

k_{PAE} = rate constant for adsorbed ^{3}H-peptide hydrolysis

R = (activity in insoluble biomass) (^3H_2O + biomass activity)$^{-1}$

Values of k_1, k_2, k_3, R, k_P, k_a, and k_{PAE} were estimated by fitting the equations to the data (peptide and FAA activity in pore water, acid extracts, and ^3H_2O) simultaneously (for detail see Chapter 2). The best-fit lines are shown in Figure 3.5. The rate constants of ^{3}H-peptides and ^{3}H-FAAs are shown in Table 3.1. The values of the hydrolysis rate constants (k_P) of ^{3}H-peptide decreased in the order: ala_2 > glu_2 > lys_2 > ala_6. The k_P values calculated using the tracer model are close to those calculated using the diffusion model (k_d) and by direct measurement (k_{dm}). Comparing the hydrolysis rate constants of ^{3}H-peptides (k_P) with the mineralization rate constants of the hydrolyzed ^{3}H-amino acid (k_1), k_1 is 2 to 6 times greater than k_P except that k_1 is equal to k_P for ^{3}H-lys_2. This reflects the accumulation of the ^{3}H-lysine in pore water for 5 to 6 hours during the ^{3}H-lys_2 decomposition (Figure 3.25).

For the three ^{3}H-FAAs, the values of the respiration rate constants (k_1 in units of h^{-1}) are: 1.30 - 2.0 (alanine), 1.20 (glutamic acid), 0.40 (lysine). This (except for lysine) is consistent with the results of Sugai and Henrichs (1992) using a similar model of the data from studies of the decomposition of ^{14}C-amino acids: 1.20 - 1.80 (alanine), 0.66 - 1.08 (glutamic acid), 1.80 - 8.40 (lysine). However, the values of the adsorption rate constant (k_3 in units of h^{-1}) calculated by the model are 5 to 10 times smaller than those calculated by Sugai and Henrichs (1992). These differences reflect some obvious

quantitative differences in the data. Far less ^{3}H-lysine, glutamic acid, or alanine were present in the acid extracts than was found by Sugai and Henrichs (1992). The most likely explanation is that only relatively small amounts of ^{3}H-FAA accumulated in pore water in my experiments, so that only limited adsorption of FAA could occur. There are two possible reasons for the low accumulations of ^{3}H-FAA. The first is that ^{3}H-peptides were assimilated directly by bacteria. The second is that the rate of peptide hydrolysis was less than that of amino acid uptake by bacteria plus adsorption, which is the result obtained using the tracer model (Table 3.1), which assumed that peptides were hydrolyzed before the constituent amino acids were taken up by bacteria. The second reason appears more likely, based on the observation that very little ^{3}H-alanine accumulated in pore water due to ala_6 hydrolysis; ala_6 is probably too large a molecule to taken up directly by bacteria. Peptide hydrolysis and amino acid uptake appear to be closely coupled in Resurrection Bay sediments, as is often the case in seawater (Hollibaugh and Azam 1983).

From the rate constants calculated using the tracer model, the rates of hydrolysis and respiration can be calculated if the concentrations of peptides and amino acids in pore water are known. However, it is very difficult to measure the actual concentration of a particular peptide in pore water by HPLC. So, as in Chapter 2, I assumed the peptide concentration in pore water was between the added concentration (C_{added}) and the concentration of peptide-like material (C_P^*). Both C_P^* and C_{added} were used for the alternative calculations. For the four peptides shown in Table 3.2, the calculated hydrolysis rates of peptides are equal to or less than the respiration rates of the free amino acids, which indicates the hydrolysis is the rate-limiting step of peptide decomposition in Resurrection Bay sediment, as it was in Skan Bay sediment.

Table 3.2. Decomposition Rates[1] of Peptides in Resurrection Bay Sediments

peptide	hydrolysis rate[2] (nmol cm^{-3} d^{-1})		respiration rate[3] (nmol cm^{-3} d^{-1})
	$24\phi(k_P+k_a)C_P^*$	$24\phi(k_P+k_a)C_{added}$	
di-alanine	5.81 - 21.19	0.75	7.20 - 64.80
hexa-alanine	15.94 - 39.23	0.15	3.51 - 31.59
di-glutamic acid	14.92 - 15.99	0.39	1.08 - 86.4
di-lysine	1.35	0.29	0.36 - 0.72

[1] The rate constants calculated using the tracer model were used in the rate calculation.

[2] Hydrolysis rate = $24\phi(k_P+k_a)C_P^*$, in which ϕ is 0.625, the sediment porosity (pore water volume/sediment volume); k_p is the hydrolysis rate constant; C_P^* is the peptide concentration in pore water, 0.31 - 1.13 μM for di-alanine-like material, 4.25 - 10.46 μM for hexa-alanine-like material, 1.53 - 1.64 μM for di-glutamic acid-like material, and 0 - 0.164 μM for di-lysine-like material. C_{added}, 0.03 μM, is the concentration of the added peptide.

[3] Respiration rate = $24\phi(1-R)k_1C_{FAA}^*$. k_1 is the respiration rate constant of the FAA; C_{FAA}^* is the concentration of the FAA in pore water, 0.30 - 2.70 μM for of ^{3}H-FAA activity taken up by bacteria which is incorporated into insoluble biomass.

Adsorption of peptides In untreated sediment, the rate constants of ^{3}H-peptide adsorption (k_a in units of h^{-1}) calculated using the tracer model (Table 3.1) were not consistent with observations (Figure 3.10), in terms of the time needed for maximum adsorption. The tracer model probably underestimated the adsorption rate, because the model is not sensitive to peptide adsorption, since the amount adsorbed is very small compared to that hydrolyzed.

The peptide adsorption data is linear with concentration on a log-log plot (Figure 3.27) and fits the Freundlich equation:

$$V_{ads} = \kappa V_{diss}^{\frac{1}{n}}$$

where V_{ads} is the adsorbed concentration (nmol/g dry sediment), V_{diss} is the dissolved concentration (nmol/mL). κ is related to the partition coefficient (adsorption extent) and binding energy between the surface and the sorbate. 1/n is related to the curvature of the adsorption isotherm. When 1/n is close to 1, κ (the intercept) is in units of mL/g dry sediment and is equal to the partition coefficient at 1 nmol/mL concentration.

The parameters for the data in Figure 3.27 are: κ = 0.19 and 1/n = 1.09 (r^2 = 0.982) for lys_2; κ = 0.08 and 1/n = 0.98 (r^2 = 0.995) for glu_2; κ = 0.16 and 1/n = 0.93 (r^2 = 0.920) for ala_2; κ = 0.19 and 1/n = 0.87 (r^2 = 0.993) for ala_3; and κ = 0.21 and 1/n = 1.02 (r^2 = 0.987) for ala_6. The value of 1/n for all five peptides is near 1, which indicates that all adsorption sites on the sediment are similar. As for Skan Bay sediment, the adsorption of lys_2 is greater than that of glu_2, ala_2, and ala_3. But, in contrast to Skan Bay, adsorption of alanyl peptides increases with molecular weight in Resurrection Bay sediment, so that ala_6 is adsorbed more than lys_2.

The 1/n values of lys_2 and ala_6 are greater than 1, while those of the other three peptides are less than 1. This reflects the observation that the adsorbed proportions of lys_2 and ala_6 increased slightly with concentration while that of glu_2 decreased (Figure

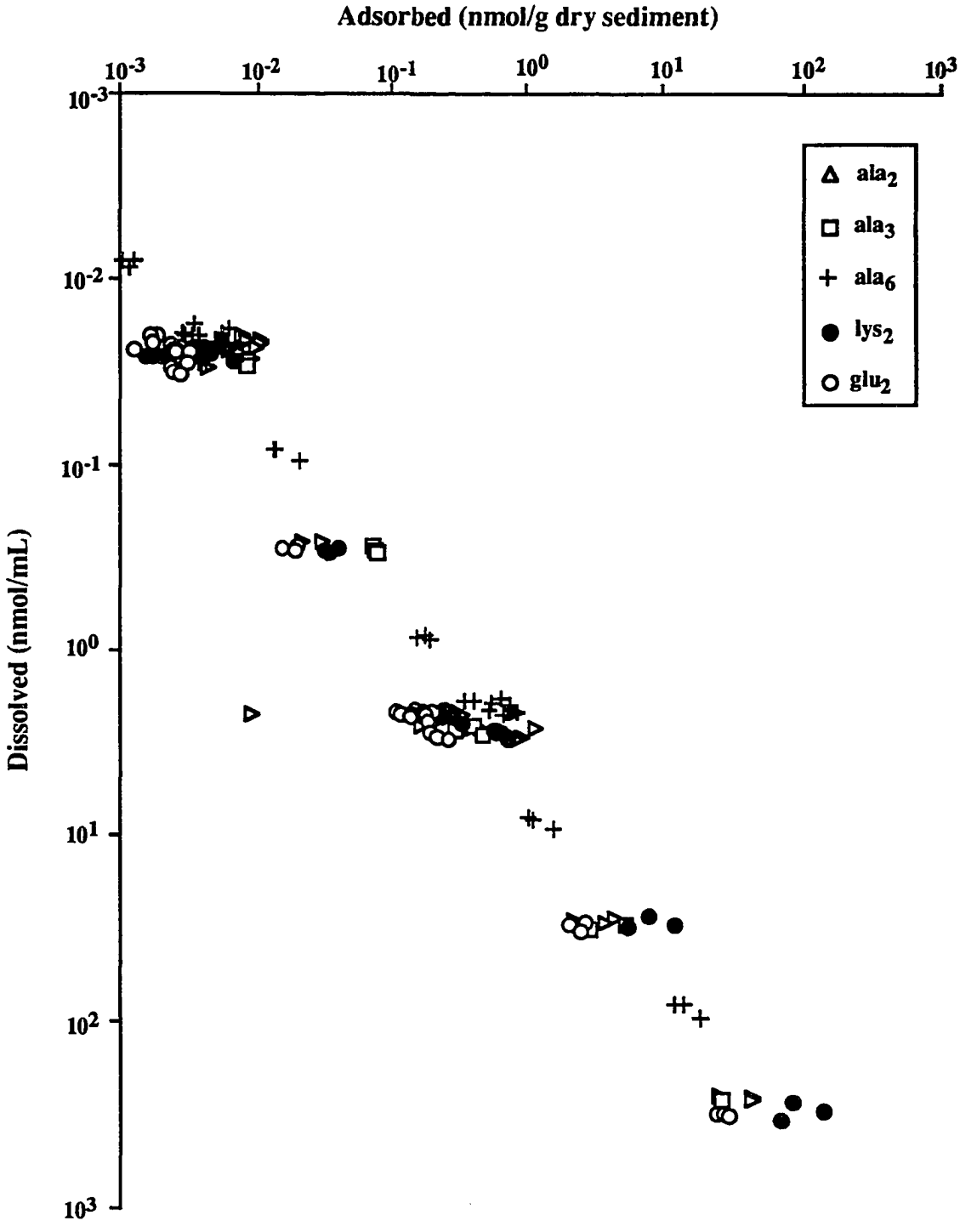


Figure 3.27. Adsorption Isotherms of ^{3}H-Peptides in Resurrection Bay Sediments.

3.11). Ala_3 adsorption did not show a consistent trend with concentration. As discussed in Chapter 2, $1/n > 1$ may indicate competition for adsorption sites by amino acids or peptide-like substances in pore water at low concentrations. $1/n < 1$ indicates that at least two kinds of adsorption sites, with slightly different peptide affinities, are present.

According to the data in Figure 3.27, adsorption of peptides increased nearly linearly with concentration over the entire range of concentrations examined. The lack of evidence for saturation of adsorption sites means that the sediment has a very large adsorption capacity for these peptides, compared to the pore water concentration. The concentration of peptide-like compounds was between 0.16 and 10.5 μM.

Previous studies on the adsorption of free amino acids and peptides by kaolinite and montmorillonite found that clay minerals could be one possible source of adsorption sites (e.g., Dashman 1977; Hedges and Hare 1987). However, no montmorillonite and very little clay, only 2 - 3% of chlorite, is present in Thumb Cove sediment (Doyle 1988). Thus, it is unlikely that clays provide most of the adsorption sites. The other possible source of adsorption sites is the acidic functional groups of sediment humic and fulvic compounds. These substances are known to adsorb cations (Rashid 1985). Thumb Cove sediment contains about 500 nmol of C per g dry weight (Henrichs and Sugai 1993). As one nmol of lys_2 has 12 nmol of C, about 1200 nmol of C in lys_2 was adsorbed at 333 μM. For the other four peptides, the maximum adsorption was between 200 and 400 nmol of C per g dry weight.

In the pretreatment experiment, cation-exchange rather than anion-exchange was identified as an important mechanism of peptide adsorption, so negatively-charged functional groups of the sediment organic matter are presumably the primary adsorption sites. However, the number of negative functional groups in the sediment would certainly be smaller than the number of positively charged groups on the adsorbed lys_2

at high concentration. Henrichs and Sugai (1993) reached a similar conclusion about the adsorption of lysine in this sediment. They proposed that lysine-lysine binding occurred at high concentrations. If this is correct, it is likely lys_2-lys_2 binding occurred. The 1/n value of lys_2, calculated by Freundlich equation, is greater than 1 which may indicate that lys_2-lys_2 binding is stronger than lys_2-particle binding in sediments.

The size, functional groups, and shape of amino acids and peptides are important factors controlling adsorption (Dashman 1977). Basicity is a particularly important factor. Increasing the number of basic functional groups (-NH_2) and the intramolecular distance between the -COOH group and the -NH_2 group makes amino acids and peptides more basic. Thus, the adsorption of both lysine (Henrichs and Sugai 1993) and lys_2 was greater than that of the other amino acids or peptides studied. It has been previously reported that adsorption is enhanced by increasing molecular weight for the adsorption of peptides and amino acids to clay minerals (e.g., Greenland *et al.* 1962, 1965a and b; Dashman and Stotzky 1984). The adsorption capacity (κ) of Thumb Cove sediments did partly follow this prediction: $ala_6 \geq lys_2 \geq ala_3 \geq ala_2 > glu_2$. The exception was the low glu_2 adsorption. However, the difference in adsorption among these peptides (except for glu_2) was not significant. The basicity of alanyl and glycyl peptides does not increase with increasing molecular weight, based on their apparent dissociation constants measured in distilled water (Cohn and Edsall 1943). Thus, the relatively greater extent of adsorption of lys_2 and ala_6 at high concentration was probably due to peptide-peptide binding.

The adsorption of peptides in seawater-rinsed sediments was different from that in untreated sediments (Figure 3.18). The seawater rinse could decrease the amino acid concentration in pore water or remove amino acids and $-NH_3^+$ from adsorption sites. Both would be likely to increase the peptide adsorption, but an increase was only found

for ala_2 adsorption. Preadsorption of a cation exchanger (Cs^+) reduced the adsorption of glu_2 and lys_2 to the same extent as the seawater rinse did. It is possible that both seawater cations and Cs^+ occupied adsorption sites of peptides. However, the cation concentration in seawater was very low compared to that of Cs^+, which had a concentration, in the sediment slurry, of 0.48 M. So, it seems unlikely that seawater cations were solely responsible for the decreased adsorption. Henrichs and Sugai (1992) also found a decrease in adsorption of amino acids in seawater-rinsed sediment but could not explain their results. Wang and Lee (1993) reported that adsorption of amino acids, especially alanine and glutamic acid, decreased in seawater-extracted sediment. They proposed that this was due to the loss of organic substances of sediments during the extraction, and that the sediment organic matter played an important role in adsorption of amino acids. Since there is little clay in Resurrection Bay sediments, organic matter is probably the primary adsorber, and its extraction by CsCl solution or seawater may explain my observation.

Comparing the peptide adsorption in pretreated and non-pretreated sediments (Figure 3.18), 50% of the adsorption of lys_2 and glu_2 is due to cation exchange, because Cs^+ pretreatment blocked about 50% of the adsorption. Similarly, 40% of the adsorption of ala_2 and ala_6 is by cation exchange. Citrate could neither block nor exchange any adsorbed peptides. The reason that preadsorption of citrate anion increased the adsorption of peptides (Figure 3.18) could be that citrate has three -COOH groups in its molecular structure, and the -COOH groups which were preadsorbed onto the sediment became new adsorption sites; thus, the adsorption of peptides, especially glu_2 and ala_2, was enhanced.

The similar extraction of adsorbed lys_2 and glu_2 by NaOH and HCl (Figure 3.14) could indicate that carboxylic groups are responsible for adsorption in addition to amino

groups. H^+ has great affinity for weak carboxylic acid sites while NaOH could act in part by the deprotonation of amino groups at high pH. However, both base and acid solutions extract fulvic acids from sediments. Thus, it is possible that lys_2 and glu_2 were adsorbed by sedimentary fulvic acids.

Some peptide adsorption was not reversible by cation exchange (Figures 3.14). The amount of irreversible adsorption was larger than would be expected based on the pretreatment experiment. For long adsorption times, some of the adsorbed peptides are not extractable even by strong acid (Figure 3.15). This suggests that additional processes or chemical reactions occur, soon after the formation of ionic bonds, which render adsorption irreversible. One evidence of this was that the peptide adsorption in pretreated sediments was less reversible than that in untreated sediments (Figure 3.19). Not only amino acids, but also a wide variety of organic and inorganic pollutants, have been found to be adsorbed irreversibly to sediments (e.g., Di Toro and Horzempa 1982; Di Toro *et al.* 1986; Henrichs and Sugai 1993; Wang and Lee 1993).

Melanoidin-type condensation reactions, which involve condensations between aldehydes and amino acids, have been proposed as a pathway of the formation of sedimentary humic and fulvic acids (Nissenbaum 1974; Krom and Sholkovitz 1977). Hedges (1978) reported that the basic amino acid lysine reacted with glucose much more rapidly than glutamic acid (acidic) or valine (neutral) to form melanoidin-type polymers. However, I found that the irreversible adsorption of lys_2 was not substantially different from that of neutral and acidic peptides. As yet, I cannot identify the processes leading to irreversible adsorption.

Adsorption and decomposition In sediments, adsorption and decomposition are two competing processes in the removal of peptides from solution. When enzymatic activity is high, more peptides will be hydrolyzed rather than adsorbed;

the product amino acids may then be adsorbed or decomposed. For example, in Figure 3.8, which shows results for stored sediments, the hydrolysis of ^{3}H-glu$_2$ and ala$_2$ was so rapid that a substantial amount of the added peptides were hydrolyzed. Thus, adsorption of these peptides was lower in the stored sediments than that in fresh sediments (APPENDIX III, Table A.4). Doyle (1988) and Sugai and Henrichs (1992) found that adsorption of amino acids in Resurrection Bay sediment was rapid and resulted in a marked decrease in the decomposition rate, at least on time scales of hours to weeks.

The hydrolysis rate constants of dissolved peptides (k_P) were much greater than those of adsorbed peptides (k_{PAE}) (Table 3.1). Although the proportion of peptide adsorption is small compared to that of hydrolysis, once peptides are adsorbed, they are less likely to be hydrolyzed. Several studies in seawater concluded that adsorption, especially irreversible adsorption, could protect peptides from further biological degradation (e.g., Samuelsson and Kirchman 1990). This study also found that adsorbed ^{3}H-peptides (lys$_2$ and ala$_6$) were quite stable over a 48-hour incubation (Figures 3.28 and 3.29).

Conclusions

1. ^{3}H-peptides (ala$_2$, ala$_6$, lys$_2$ and glu$_2$) at 0.03 μM initial concentration were not hydrolyzed or respired in pore water that had been filtered through a 0.2 μm filter. Hydrolytic enzyme activity was associated with the sediment or bacteria.

2. In sediments, ^{3}H-peptides were largely hydrolyzed after only 1 hour. The hydrolysis rate constants of ^{3}H-ala$_2$ and glu$_2$ were greater than those of ^{3}H-lys$_2$ and ala$_6$. The rate of respiration of peptides to ^3H_2O varied in the order: ala$_2$ > ala$_3$ > glu$_2$ > ala$_6$ > lys$_2$. Hydrolysis is the rate-limiting step of peptide decomposition, because rates of ^{3}H-peptide hydrolysis were close to those of ^{3}H-FAA respiration.

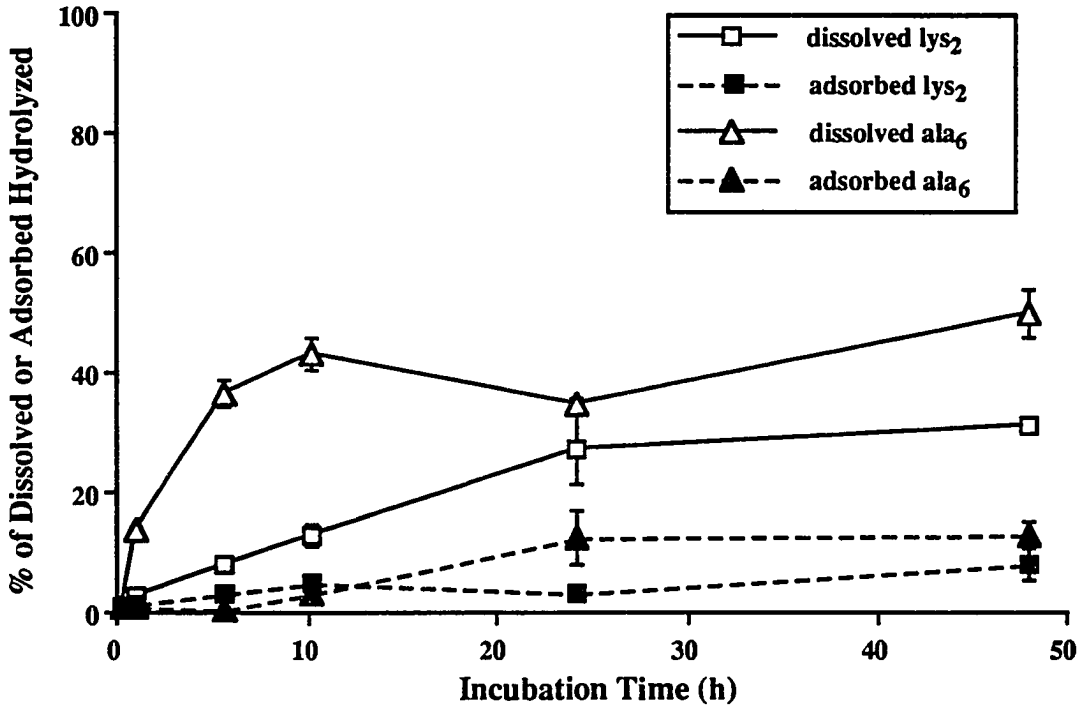


Figure 3.28. Hydrolysis of Dissolved and Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Resurrection Bay Sediments.

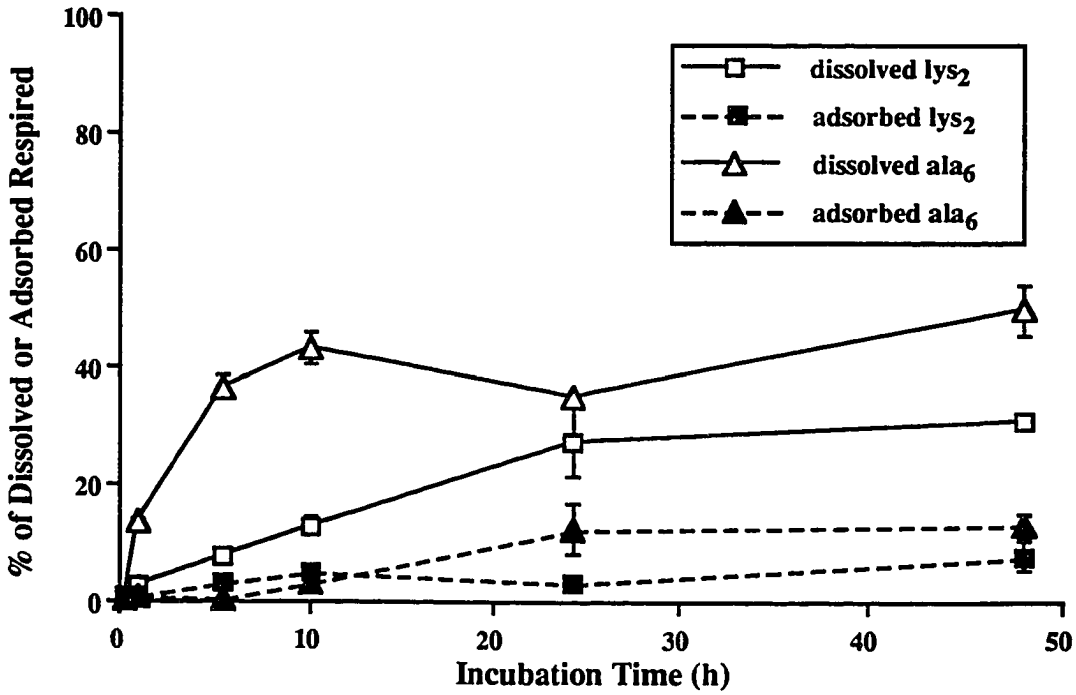


Figure 3.29. Respiration of Dissolved and Adsorbed ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Resurrection Bay Sediments.

3. Adsorption of ^{3}H-peptides in fresh and autoclaved sediments was similar and appear to increase during the first 1 hour. The adsorption partition coefficients on sediment were: 0.21 (ala_6), 0.19 (lys_2), 0.19 (ala_3), 0.16 (ala_2), 0.08 (glu_2) mL/g dry sediment.

4. Adsorbed ^{3}H-lys_2 and ala_6 decomposed slowly. For ^{3}H-ala_2 and glu_2, the rate of hydrolysis is greater than that of adsorption, so adsorption has a smaller effect on decomposition of these two peptides.

5. Part of the ^{3}H-peptide adsorption (approximately 50% of glu_2 and lys_2, and 40% of ala_2 and ala_6 adsorption) was identified as being via cation-exchange. Some of the adsorption was not reversible.

Chapter 4: Decomposition, Adsorption, and Preservation of Peptides in Marine Sediments

Introduction

Early diagenesis of organic matter in marine sediments is the combination of biological, chemical, and physical processes which change the quantity and composition of the organic matter in the upper several hundred meters of the sediment column. Although from 30% to more than 99% of incoming organic matter is remineralized within the upper meter of marine sediments, some of the organic matter deposited on the sediment surface survives (Henrichs 1992). Much of the preserved organic matter consists of humic substances (Rashid 1985) or refractory biopolymers (Tegelaar *et al.* 1989), but intrinsically labile compounds such as lipids or hydrolyzable amino acids are also present. Some organic substances with a clearly biological origin have survived a billion years in sediments (Mycke *et al.* 1988; Summons *et al.* 1988). Because most of the transformation and mineralization of organic matter occurs within 1 m of the sediment surface, processes in this zone are likely the key to the mystery of preservation.

The extent to which organic matter is preserved in marine sediments is highly variable geographically, and the reasons for this variation are still controversial. One hypothesis is that anoxic or low-oxygen conditions favor the preservation of organic substances (Emerson and Hedges 1988; Canfield 1989). This is because benthic bacteria and animals are largely responsible for early diagenesis; animals are absent under highly anoxic conditions, and individual species of anaerobic bacteria generally catabolize only a restricted range of organic molecules. However, evidence that anoxic conditions are mainly responsible for enhanced organic preservation is disputed (Calvert and Pedersen 1992). Another hypothesis concerning organic matter preservation is that the surviving

residue consists of intrinsically refractory biopolymers (Tegelaar *et al.* 1989). Alternatively, some studies have suggested that adsorption of dissolved molecules by sediment humic substances or clay minerals could be a key process in their preservation (e.g., Marshman and Marshall 1981; Henrichs and Sugai 1993; Mayer 1994). However, for most molecules, the chemical reactions that occur during their adsorption to natural sediments, as well as the effect of adsorption on bioavailability and the rates of decomposition, are still unknown (Henrichs 1992).

Hydrolyzable amino acids are the largest identifiable fraction of the nitrogen-containing organic matter preserved in marine sediments. In this chapter, the aerobic and anaerobic decomposition of adsorbed and free peptides will be compared in order to understand better how the physical and chemical environment of hydrolyzable amino acids in sediments affects their diagenesis and preservation.

Factors Affecting Peptide Adsorption in Sediments

Comparison of peptide adsorption in Skan Bay and Resurrection Bay sediments Adsorption parameters of peptides in sediments from Skan Bay (SB) and Resurrection Bay (RB) are compared in Table 4.1. They were calculated by fitting the Freundlich equation:

$$V_{ads} = \kappa V_{diss}^{\frac{1}{n}}$$

As described in Chapters 2 and 3, κ is related to the partition coefficient, which indicates the binding energy between the surface and the sorbate. The values of κ are over 10 times greater in SB than in RB for di-peptides, and 1.5 to 2 times greater for ala_3 and ala_6 (Table 4.1). In both sediments, adsorption of di-peptides has the same pattern: basic > neutral > acidic. However, κ among the alanyl peptides in SB decreases with increasing molecular weight (MW): $ala_2 > ala_3 > ala_6$, while it increases slightly with increasing MW in RB:

Table 4.1. Adsorption Parameters* of Peptides in Two Marine Sediments

sediments	peptide	partition coefficient	adsorption isotherm curvature
		κ	$\frac{1}{n}$
Skan Bay	lys_2	1.20	0.985
	glu_2	0.81	1.009
	ala_2	1.16	1.154
	ala_3	0.42	1.003
	ala_6	0.31	1.057
Resurrection Bay	lys_2	0.18	1.090
	glu_2	0.08	0.983
	ala_2	0.16	0.927
	ala_3	0.18	0.866
	ala_6	0.20	1.015

* Parameters were calculated using the Freundlich equation: $V_{ads} = \kappa V_{diss}^{\frac{1}{n}}$.

$ala_2 \leq ala_3 \leq ala_6$. Thus, κ varies in the order $lys_2 > ala_2 > glu_2 > ala_3 > ala_6$ in SB, and $ala_6 > lys_2 > ala_3 > ala_2 > glu_2$ in RB.

Several studies have concluded that sedimentary organic matter (OM) plays an important role in the adsorption of amino acids, acetate and aliphatic amines in different sediments (Rosenfeld 1979; Sansone *et al.* 1987; Henrichs and Sugai 1993; Wang and Lee 1993). Most natural marine particles are negatively charged, due to surficial functional groups such as carboxylate ($-COO^-$) and phenolate ($Ø-O^-$) (Hunter 1980; Davis 1982), and these functional groups are plausible adsorption sites. SB contains 10 times more OM than RB (Alperin 1988; Doyle 1988). Although the mass ratio of organic content does not relate directly to the quantity of functional groups available at the particle surfaces for binding peptides, SB sediments should have more adsorption sites than RB. Thus, K_{ads} of peptides should be greater in SB than in RB sediments, which was the result found at higher concentrations, at which adsorption sites might be limiting.

The curvature of the adsorption isotherm is related to 1/n. When 1/n approaches 1, the adsorption is linearly related to concentration. If there are a limited number of adsorption sites, or if there are at least two different kinds of adsorption sites with significantly different affinities for sorbate, the value of 1/n will be less than 1. If the sorbate has a greater tendency to bind with itself than with the particle surface, or if there are other sorbates in solution that compete for sorption sites at low concentration, the value of 1/n will be greater than 1. If all adsorption sites are similar and the number available does not limit adsorption, the value of 1/n will be close to 1. In both SB ad RB sediments, 1/n for all peptides is close to 1, but varies among the peptides between the two sediments (Table 4.1). In SB sediment, 1/n values of all the peptides are greater than 1, except that of lys_2 is less than 1. In RB sediment, 1/n values of lys_2 and ala_6 are greater than 1 while those of glu_2, ala_2 and ala_3 are less than 1. This corresponds to the variation

of the partition coefficient (K_{ads}, in units of mL/g dry sediment) with concentration shown in Figure 4.1. K_{ads} is calculated from the following equation:

$$Kads = \left(\frac{\% \text{ adsorbed}}{\text{g dry sediment}} \Big/ \frac{\% \text{ dissolved}}{\text{mL solution}} \right)$$

Differences between SB and RB sediment properties mostly affect adsorption at high concentrations, or if the peptide has extra $-NH_3^+$ or $-COO^-$ functional groups. For lys_2, K_{ads} in SB decreased when the concentration increased to 1,000 nmol/mL, while in RB, it increased with concentration, so that the values in SB and RB were about the same at the highest concentration tested (Figure 4.1). This trend is shown by the 1/n values also: $1/n < 1$ in SB, $1/n > 1$ in RB (Table 4.1). The data indicate that sedimentary OM in SB strongly adsorbs lys_2, while lys_2 in RB sediment may prefer binding with itself rather than to the particle surfaces. Henrichs and Sugai (1993) suggested lysine-lysine binding occurred in RB sediments at high lysine concentrations (e.g., 1 - 10 mM) because there were more functional groups in the adsorbed lysine molecules than in the sedimentary OM, which is probably also the case for lys_2. Similar, naturally occurring molecules dissolved in pore water, e.g., free amino acids and peptides with the same terminal amino groups, may compete with added peptides for adsorption sites in sediments. In pore waters from both SB and RB, the concentrations of lys_2-like peptide and lysine are similar (Table 4.2), and so is the concentration of hydrolyzable lysine (MW < 850, 000) (Table 4.3). Thus, concentrations of dissolved peptides and amino acids in pore water are unlikely to be responsible for the differences in lys_2 adsorption in the two sediments.

The 1/n of glu_2 in RB and SB was closer to 1 than the values for other peptides (Table 4.1). This indicates that all adsorption sites on sediment surface for glu_2 are similar. The concentrations of glutamic acid and glu_2 like peptides in RB are greater than

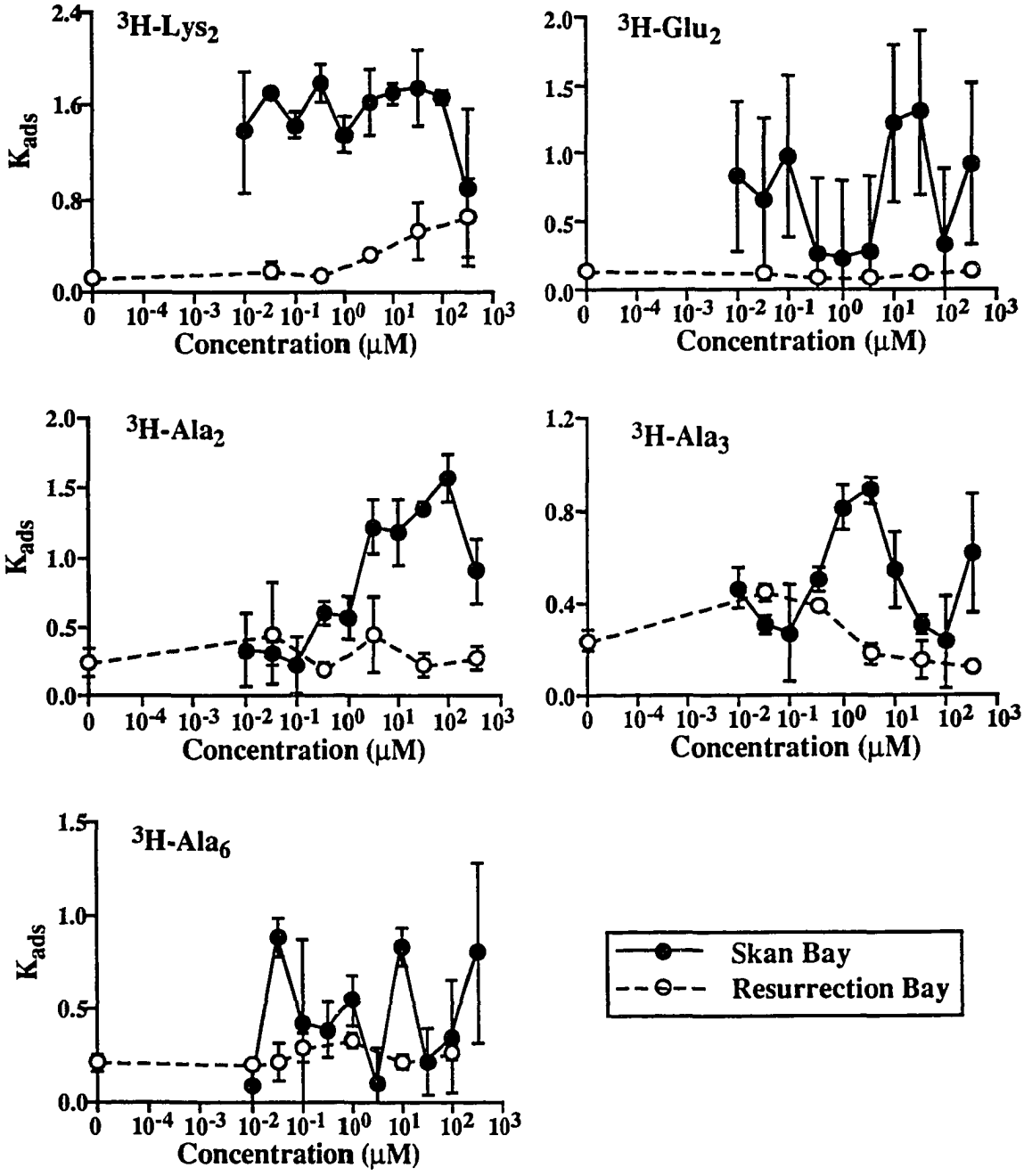


Figure 4.1. Adsorption Partition Coefficients (K_{ads}) of Peptides in Two Sediments. Skan Bay sediment was autoclaved. Resurrection Bay sediment was untreated.

Table 4.2. Concentrations of Peptides and Amino Acids in Pore Waters from Two Marine Sediments

peptide/amino acid	concentration (μM)		reference
	Skan Bay	Resurrection Bay	
di-lysine	0.13 - 1.79	n.d. - 0.16	This study
lysine		n.d.	Doyle 1988
	0.06	0.2	McDaniel 1989
		0.1±0.1	Henrichs and Sugai 1992
di-glutamic acid	1.63 - 2.45	1.54 - 1.60	This study
glutamic acid		8.0±3.7	Doyle 1988*
	0.13 - 0.41	0.2 - 1.8	McDaniel 1989
		3.7±0.7	Henrichs and Sugai 1992
	0.3±0.09	0.84 - 3.02	This study
di-alanine	n.d.	0.31 - 1.13	This study
tri-alanine	n.d. - 0.62		
hexa-alanine	n.d. - 2.45	4.25 - 10.5	
alanine		2.7±1.7	Doyle 1988*
	0.39 - 0.46	0.3 - 2.3	McDaniel 1989
		1.6±0.5	Henrichs and Sugai 1992
	0.24±0.13	0.59 - 2.08	This study

*Samples were taken from the 0 - 1 cm depth interval, which is oxic and has high amino acid concentrations.

Table 4.3. Amino Acid Concentrations from Hydrolyzable Peptides in Pore Water[1]

amino acid	concentration (μM)			
	Resurrection Bay		Skan Bay	
	sample 1	sample 2	sample 1	sample 2
glutamic acid	1.14	-	3.80	4.57
β-glutamic acid[2]	-	0.73	3.85	0.92
alanine	0.11	1.63	0.24	0.21
β-alanine[3]	0.34	0.37	2.64	1.55
lysine	0.20	-	-	-
glycine+threonine	0.61	0.27	4.43	1.78

[1] Organic compounds in pore water were separated according to molecular weight by size-exclusion HPLC using a Synchropak GPC 200 column. The fraction (MW < 850,000) was collected and hydrolyzed for combined amino acids. Hydrolysis was conducted in 6N HCl, at 110°C for 20 hours (Robertson *et al.* 1987). OPA derivatives of the hydrolyzed amino acids were analyzed by reversed-phase HPLC using a Lichrosorb RP-18 column. The concentration of each hydrolyzable amino acid has been corrected for the concentration of free amino acids in the fraction.

[2] The concentrations of β-glutamic acid and β-alanine have been overestimated because their peaks cannot be completely separated on HPLC from the major peaks of glutamic acid and alanine, respectively.

or equal to those in SB pore water (Table 4.2). Pore water composition has no apparent effect on glu_2 adsorption.

For the alanyl peptides, K_{ads} in SB was greater than that in RB for each peptide at concentrations ≥ 0.1 nmol/mL, and the differences between these two sediments were greater for ala_2 than for ala_3 and ala_6. However, K_{ads} in SB was similar to that in RB at concentrations ≤ 0.1 nmol/mL; K_{ads} was about 0.2 mL/g dry sediment. Thus, K_{ads} did not change or decreased with increasing concentration in RB sediments, while K_{ads} increased with concentration in SB sediments. Corresponding to this, 1/n for the alanyl peptides in SB sediments was greater than 1, while in RB it was less than one (except for ala_6). The concentrations of alanyl-like peptides in RB pore water are greater than those in SB pore water, while the concentration of alanine in RB is greater than or equal to that in SB pore waters (Table 4.2). If dissolved peptide concentrations affect adsorption, this would occur in RB rather than in SB. Thus, it is more likely that the trends are due to alanyl peptides preferentially binding to themselves rather than to SB sediment surfaces.

Larger peptides or protein dissolved in pore water might also affect the adsorption of small peptides, as they could compete for adsorption sites. The composition of the dissolved hydrolyzable amino acids differed in two sediments, with more glutamic acid, glycine and threonine but less alanine found in SB pore water compared to RB pore water (Table 4.3). If the total concentration of hydrolyzable amino acids strongly affects adsorption, adsorption should be greater in RB than in SB sediments at low added peptide concentrations. The actual adsorption did not follow this pattern: peptide adsorption in SB was greater than or equal to that in RB sediment (Figure 4.1). Differences in adsorption between the two sediments are principally due to differences in properties of the sediment particles.

Greater adsorption per unit mass is expected for small size particles, because of their large surface areas, than for large ones (Mayer and Rossi 1982). Wang and Lee (1993) investigated the adsorption of aliphatic amines and amino acids by coastal sediment of various particle sizes. They found that the smallest particles (< 65 μm) adsorbed the most for all the compounds tested. Studies of DNA adsorption on different kinds of soils (Ogram *et al.* 1993) also showed that particle size played an important role in controlling adsorption. The adsorption patterns on particles of different sizes were different. In organic-free sand, the adsorption of DNA increased with polymer length because there were more sorptive moieties in longer polymers. But in soils with the texture of silty clay, there was an inverse relationship between DNA size and adsorption extent due to the size-exclusive effect of clay on DNA above a certain polymer length. However, none of the peptides I investigated is big enough for size exclusion to occur.

A very strong relationship between total OM concentrations and sediment grain size, particularly in shallower marine environments, has been reported by Premuzic *et al.* (1982). Clay minerals and organic matter are enriched in the smallest size range (Meyers and Quinn 1973; Folk 1975). Because of these associations, it is difficult to separate the adsorption effects of clays and organic matter. Another reason that it is difficult to consider the effects of organic matter and clays separately is that natural particles are coated with OM adsorbed from seawater while sinking through the water column, so that further adsorption is a heterogeneous process, with attachment to organic molecules already adsorbed on the surface (Mayer 1994). OM coatings on particle surfaces play a major role in their surface chemistry by changing the electrochemical potential, CST, and hydrophobicity and hydrophilicity (Hunter and Liss 1979; Tipping and Cooke 1982; Loder and Liss 1985).

Wang and Lee (1993) reported that the adsorption of lysine in oxidized sediment was much higher than that in anoxic sediment. They related the increase of adsorption to the oxidation of reduced metal species, e.g., FeS to FeOOH and MnS to MnOOH. The oxides would provide more surface area for adsorption in the sediment (Sigg and Stumm 1981; Davis 1982). However, I saw no evidence of enhanced adsorption in the more oxic RB sediment. The clay in RB sediment consists primarily of Fe-rich chlorites (Doyle 1988), but clay minerals are not likely the major adsorber because they make up only 2-3% (dry weight) of the sediment (Doyle 1988). Skan Bay sediment contains < 6% clay, consisting of 78% chlorite, 22% illite, and a trace amount of kaolinite. Among the different clay mineral constituents, chlorite and illite are very weak absorbers compared with kaolinite and montmorillonite. By comparing the adsorption extent of amino acids on RB sediment with that on organic-free kaolinite and montmorillonite (Hedges and Hare 1987), Henrichs and Sugai (1993) concluded clays were not the major adsorbers for amino acids in RB sediment because of their low abundance. I draw a similar conclusion about clays in both SB and RB sediments, both because of low clay content and because the relative levels of adsorption are not proportional to the relative clay content.

The actual, *in situ*, distribution of peptides between the dissolved and particulate phase of sediments depends on sediment porosity, the pore water to particle volume ratio. SB sediment porosity (ϕ) is 0.88 while that of RB is 0.63, and so SB contains less particulate matter per volume of sediment than RB. In order to eliminate the effect of sediment porosity, a porosity-independent, or dimensionless coefficient (K) can be used. K is the dimensionless ratio of adsorbed/dissolved peptides in the sediments.

$$K = K_{ads}\rho_s(1-\phi)/\phi$$

where K_{ads} is the partition coefficient and ρ_s is dry sediment density (1.9 for SB and 2.5 for RB). Adsorption of peptides in SB and RB sediments is more similar in terms of K

than K_{ads}. K_{ads} is most useful in discussing the properties of sediment particles, but K can make clear the importance of the adsorption process. Because of the similarity of K, adsorption effects on the fate of peptides are likely to be similar in RB and SB sediments.

In summary, adsorption of peptides by Skan Bay and Resurrection Bay sediments was perhaps more similar, especially in terms of the dimensionless partition coefficient, than expected, given substantial differences in sediment composition and, particularly, organic content. The evidence indicates that organic matter is the major adsorber of peptides, and that differences in organic content (or composition) are mainly responsible for differences in adsorption between the two sediments. No clear effects of pore water composition on peptide adsorption, for example, competition for binding sites by pore water constituents, were seen in this study.

Comparison of adsorption of peptides, amino acids and other organic compounds Comparing the partition coefficients (K_{ads}) of peptides and amino acids in SB and RB, the major difference is that the effect of concentration on K_{ads} is much greater for amino acids than for peptides (Table 4.4). For example, in SB sediment, there was no adsorption of glutamic acid and alanine at concentrations greater than 10 μM, but substantial adsorption at lower concentrations. In both SB and RB sediments, K_{ads} values of amino acids, especially those of lysine, decreased substantially with concentration over the range from 0 to 1,000 μM. In RB sediment, the K_{ads} values of glu_2 and alanyl peptides were similar in magnitude to those of glutamic acid and alanine, but the K_{ads} values of lys_2 were about 10 times less than those of lysine over the concentration range of 0 to 1,000 μM (Table 4.4).

Over the same concentration range, K_{ads} of alanine in Danish coastal sediment (Christensen and Blackburn 1980) was slightly greater than those of alanine and alanyl peptides in SB and RB. However, the K_{ads} values of amino acids in Flax Pond sediment

Table 4.4. Partition Coefficient (K_{ads})* of Peptides and Amino Acids in Marine Sediments

sediment site	peptide	concentration (μM)	K_{ads}	amino acid	concentration (μM)	K_{ads}
RB	di-lysine	0 - 1000	0.10 - 0.59	lysine	0.01 - 1000	4.5 - 2.4
	di-glutamic acid		0.07 - 0.13	glutamic acid	0.01 - 100	0.93 - 0.08
	di-alanine		0.10 - 0.30	alanine	0.01 - 1000	0.44 - 0.17
	tri-alanine		0.43 - 0.10			
	hexa-alanine		0.20 - 0.33			
SB	di-lysine	0.01 - 1000	1.49 - 0.58	lysine	0 - 100	30 - 3.0
	di-glutamic acid		0.19 - 1.35	glutamic acid	0 - 5	21 - 2.1
	di-alanine		0.39 - 1.54	alanine	0 - 10	0.91 - 0
	tri-alanine		0.19 - 0.77			
	hexa-alanine		0.39 - 0.77			
FP				lysine	0 - 0.5	128
				glutamic acid	0 - 0.3	11.0
				alanine	0 - 1.0	9.1

* K_{ads} is the partition coefficient in units of mL/g. K_{ads} values of peptides in SB and RB are from the data in Figure 4.1 (see text). K_{ads} values of amino acids in RB are from Henrichs and Sugai (1993), and in SB, from Sugai and Henrichs (in preparation). K_{ads} in FP are from Wang and Lee (1993).

(FP) measured by Wang and Lee (1993) were much greater than those in SB and RB, and so they were also much greater than the K_{ads} of peptides. Wang and Lee (1993) suggested that higher K_{ads} values of amino acids in FP than in RB may be due to the higher sedimentary OM (2.8%) in FP. However, the K_{ads} of alanine in SB is 10 times less than that in FP, despite the fact that the organic matter concentration in SB sediment is greater. Overall, the data show no correlation between amino acid adsorption and sediment organic content. Other factors, including organic matter composition, are probably important.

Several studies of the adsorption of amino acids and peptides by clay minerals found that adsorption increased with MW (e.g., Sieskind and Wey 1959; Sieskind and Wyart 1960a and b; Dashman 1977; Dashman and Stotzky 1982). They explained that the enhancement of adsorption was due to an increase in basicity due to the increasing distance between the amino and carboxyl moieties. However, the dissociation constants (pK_i, i=1 to 5) of $-NH_3^+$ actually decrease as MW increases from amino acid to peptides (Table 4.5), which indicates decreased basicity of the $-NH_2$ group. Since lys_2 has one more $-NH_2$ group than lysine, it still has a greater isoelectric point (pI) and basicity than lysine. But for peptides made up of neutral and acidic amino acids, pI values of the molecules also decrease with MW. At the pH of pore water (7 - 8), lys_2 has a net positive charge while the other peptides are negatively charged, although most of the amino groups are protonated until pH > pK. Thus, it is expected that adsorption of alanyl peptides and glu_2 should be less than or equal to that of alanine and glutamic acid based on this criterion. However, it is difficult to explain why adsorption of lys_2 is less than that of lysine. It is possible that this is due to steric effects, i.e., some of the adsorption sites are not accessible to the larger molecule.

Although the adsorption coefficients of different peptides and amino acids measured in different marine sediments are different because of the different sedimentary

Table 4.5. Apparent Dissociation Constants of Amino Acids and Peptides in Water[1]

amino acid/peptide	pK_1 (COOH)	pK_2 (COOH)	pK_3 (NH_3^+)	pK_4 (NH_3^+)	pK_5 (NH_3^+)	pI[2]
lysine	2.18		8.95	10.53		9.74
di-lysine	1.95		8.17	9.45	10.63	10.04
glutamic acid	2.16	4.25	9.67			3.22
di-glutamic acid	3.14	4.38	7.62			-
alanine	2.34		9.69			6.00
di-alanine	3.17		8.42			5.79
glycine	2.34		9.60			5.97
di-glycine	3.06		8.13			5.60
tri-glycine	3.26		7.91			5.59
tetra-glycine	3.05		7.75			5.40
penta-glycine	3.05		7.70			5.38
hexa-glycine	3.05		7.60			5.32
gly-di-ala-glycine	3.30		7.93			5.62

[1] Cohn and Edsall (1943).

[2] pI is the pH of the isoelectric point. At pH values below the pI of the amino acid or peptide, the compound is a cation. That is, the amino moiety is - NH_3^+, the carboxylic moiety is predominantly -COOH, and the net charge of the molecule is positive. Conversely, at pH values significantly above the pI, the amino group is in mainly the $-NH_2$ form, the carboxyl group is in the $-COO^-$ form, and the net charge of the molecule is negative.

OM content and other factors, those with more basic functional groups ($-NH_2$) are generally strongly adsorbed compared to the others. Basicity plays a more important role than MW in enhancing the adsorption of peptides, and the basicity is increased by adding basic functional groups to the peptide chain rather than by adding $-CH_2-$ groups.

Adsorption mechanisms of peptides in sediments The molecular structure of peptides is important to the adsorption mechanism. The adsorption mechanism of peptides is expected to be between that of amino acids and protein. The adsorption of small peptides in clays was found to be similar to that of amino acids (Talibudeen 1955; Sieskind and Wey 1959; Greenland *et al.* 1965a and b; Weiss 1969; Dashman and Stotzky 1982). Both peptide and amino acid adsorption were enhanced by increasing MW and basicity of molecules. For proteins, the electrochemical potential, critical surface tension (CST), and hydrophobicity of the particle surface become important in controlling the adsorption (e.g., Kirchman *et al.* 1989; McGuire and Krisdhasima 1991; Taylor *et al.* 1994). Kirchman *et al.* (1989) reported that, in a high salinity solution (e.g., seawater), adsorption of protein to a highly hydrophobic surface was greater than that to a less hydrophobic surface. Magnetic resonance studies of polypeptide adsorption on silica and hydroxyapatite surfaces (Fernandez *et al.* 1992) found that poly-L-lysine and poly-L-glutamic acid adsorbed in a flat, relatively extended conformation through strong electrostatic interactions of the side-chain functional groups with the surface.

In pore water, ala_6 is less soluble (i.e., it is more hydrophobic) than the other peptides investigated, due to its long hydrocarbon chain. Adsorption of hydrophobic organic molecules to sediments and soils generally increases with sediment organic content (Stumm 1987). The adsorption of ala_6 should be favored in SB sediments, from the perspective of its hydrophobicity. Also, if hydrophobic interaction were an important mechanism in the adsorption of small peptides, ala_6 adsorption should be greater than

adsorption of other peptides, and certainly greater than that of ala_2 and ala_3. This was not observed in SB sediment and was only weakly true in RB sediment. So, hydrophobic interactions are not important in the adsorption of small peptides.

Pretreatment and extraction experiments (e.g., Wang and Lee 1993; this study) were consistent with the interpretation that the sedimentary OM coating on particle surface is the major adsorber of amino acids and peptides. For amino acids and peptides adsorbed by SB and RB sediments, extraction by the ion-exchange solution cesium (chloride) (CsCl) and hydrochloric acid (HCl) was similar (Figures 2.22, 2.23 and 3.14; Henrichs and Sugai 1993). Ionic attraction between the protonated amine groups of peptides and negatively-charged groups of sedimentary OM appears to be involved in the adsorption. However, part of the adsorption is irreversible by seawater and ion-exchange solutions and even HCl solution, as well as by sodium hydroxide (NaOH) solution. Only acid hydrolysis could eventually release all of the adsorbed material. Thus, it is likely that some of the adsorbed peptides were bonded to sedimentary OM by a covalent rather than ionic bonds. Some experiments with RB sediments have supported the hypothesis that condensations between amino acids and aldehydes (sugars) occur, because treatment with reagents reactive with aldehydes significantly decreased lysine adsorption (Henrichs and Sugai 1993a). These reactions between amino groups and aldehydes are not reversible.

The irreversible adsorption of peptides showed a tendency to increase gradually with time (APPENDIX IV, Figures A.4 and A.6). Since the apparent adsorption coefficients did not increase substantially with time, some of the reversible adsorption became irreversible as time passed.

Aerobic and Anaerobic Processes in Sediments

In addition to adsorption, microbial decomposition is the other important sink for peptides in marine sediments. The rate of enzymatic hydrolysis of peptides is competitive with that of adsorption. The decomposition pathways of organic molecules in oxic and anoxic sediments are different. In oxic sediment, monomers released from organic molecules (such as sugars and amino acids) are oxidized directly by bacteria using oxygen as the electron acceptor, while in anoxic sediment, monomers are fermented to short-chain organic acids (including acetate), which are then oxidized to CO_2 by sulfate-reducing bacteria using SO_4^{2-} as the electron acceptor. It has been controversial whether there is an intrinsic difference between aerobic and anaerobic decomposition rates. It is difficult to answer this question by simple comparison of oxic and anoxic sediments, since they differ in many characteristics in addition to oxygen availability, including the physical and chemical environment of organic molecules.

Physical and chemical environment of organic molecules The decomposition rates of organic matter in sediments vary with structure, molecular weight and functional groups. Usually, small molecules are turned over faster than large molecules. Table 4.6 shows some rate constants of decomposition of several organic compounds in marine sediments. In both oxic and anoxic sediments, free amino acids are turned over much faster (as much as 10^4 times faster) than total hydrolyzable amino acids. The rate constants of mineralization or uptake of free amino acids and acetate are similar in magnitude to those of peptides, measured in this study. The hydrolysis rate constants of peptides gradually decreased with increasing MW. The total hydrolyzable amino acids are decomposed with the rate constants similar to those of protein and natural sediment total organic carbon (TOC). Amino acid melanoidins were decomposed as slowly as natural sediment TOC and total hydrolyzable amino acids.

Table 4.6. First-Order Decomposition Rate Constants for Organic Substances in Sediments

sediment location & type	organic substance	rate constant[1] h^{-1}	reference
Resurrection Bay, oxic surface sediment, 0.7% TOC	glutamic acid	2.17	Sugai and Henrichs 1992
	alanine	2.05	
	lysine	5.14	
	glu-melanoidin	0.2×10^{-4}	Henrichs and Doyle 1986
	ala-melanoidin	$<0.1 \times 10^{-4}$	
	natural sediment TOC	$<3.4 \times 10^{-6}$	
	glutamic acid	1.20	this study[2]
	alanine	1.3 - 2.0	
	lysine	0.40	
	di-glutamic acid	0.60	
	di-alanine	1.20	
	hexa-alanine	0.20	
	di-lysine	0.40	
Skan Bay, anoxic, 5-6% TOC	acetate	1.0	Shaw *et al.* 1984
	glutamic acid	0.05	Henrichs, unpublished
	glutamic acid	0.50	this study
	alanine	0.25 - 0.30	
	lysine	0.09	
	di-glutamic acid	1.0	
	di-alanine	0.30	
	tri-alanine	0.20	
	hexa-alanine	0.10	
	di-lysine	0.07	
Kolding Fjord, anoxic, 17% OM	free amino acids	0.3	Hansen *et al.* 1993

Table 4.6. (continued)

sediment location & type	rate constant[1] organic substance	h^{-1}	reference
Cape Lookout Bight, anoxic, 3-5% TOC	acetate	0.034	Crill and Martens 1986
	fatty acids	$0.9x10^{-4}$	Haddad 1989
	free amino acids	0.856	Burdige and Martens 1990
	total hydrolyzable amino acids	$1.6x10^{-4}$	Burdige and Martens 1988
Bordenstake Bay, anoxic, 3-4% TOC	glutamic acid	0.011 - 0.026	Burdige 1991
	alanine	0.038	
Chesapeake Bay	glutamic acid	0.031 - 0.038	
	alanine	0.112 - 0.264	
Buzzards Bay, upper 70 cm, oxic 0-2 cm, anoxic below, 1.1-2% TOC	fatty acids	$1.1x10^{-6}$	Farrington *et al.* 1977
	total hydrolyzable amino acids	$2.2x10^{-6}$	Henrichs and Farrington 1987
Lowes Cove, intertidal mudflat, 0-2 cm, about 2% TOC	protein	$1.9x10^{-4}$	Mayer and Rice 1992

[1] The rate constant was calculated by fitting concentration vs. time (or depth divided by sediment accumulation rate) data to the following equation: $G_t = (G_0-G_i)e^{-kt} + G_i$, where G_t is the concentration of the organic substance at time t, G_0 is the concentration of decomposable organic matter at t = 0, and G_i is the concentration of inert organic matter. The peptide data are hydrolysis rate constants.

[2] The rate constants were calculated using the tracer model described in Chapter 2.

The anoxic sediments from SB and Cape Lookout Bight (CLB) have similar TOC contents, and the decomposition rate constants of free amino acids in SB measured by Henrichs and this study were similar to those found in CLB by Burdige and Martens (1990). However, the rate constant of acetate mineralization measured in SB by Shaw *et al.* (1984) was almost 30 times greater than that found in CLB by Crill and Martens (1986). Although rate constants for mineralization of free amino acids in oxic RB were somewhat greater than those in anoxic SB and CLB sediments, the difference was not as large as that for acetate between the two anoxic SB and CLB sediments. Burdige (1991) reported that rate constant of alanine mineralization was greater than that of glutamic acid in Bordenstake Bay (BB) and Chesapeake Bay (CB) sediments, while the result in SB was the reverse, even though all these sediments are anoxic and contain similar amounts of TOC. Therefore, there appears to be no relationship between rate constants and oxic or anoxic conditions.

It is not clear yet how anaerobic conditions influence the activity of hydrolytic enzymes (Meyer-Reil 1991). Among the peptides investigated, peptide hydrolysis rate constants were not clearly correlated to oxic and anoxic conditions. The rate constant for glu_2 hydrolysis was greater in SB than in RB sediment. The rate constant for lys_2, ala_2, and ala_6 were of similar in magnitude but less in SB than in RB sediment (Table 4.6). The hydrolysis of ala_2 and glu_2 were dramatically greater in the pore water from anoxic SB than from oxic RB (Table 4.7). King (1986) also reported that β-glucosidase activity in intertidal marine sediments was not sensitive to the presence or absence of oxygen. But Meyer-Reil (1983) found that proteolytic enzymes in Kiel Bight sediments might be inhibited during periods of anoxia in summer. Enzymes may vary quantitatively and qualitatively for the same bacterium at different stages of growth, even under constant

Table 4.7. Free Amino Acid and Peptide Concentrations vs. Hydrolysis of Peptides in Pore Water

	amino acid/peptide	Skan Bay	Resurrection Bay
concentration	alanine	0 - 0.04 μM	0.4 - 0.8 μM
	di-alanine	0.15 - 0.17 μM	0.3 - 1.3 μM
	hexa-alanine	0.40 - 0.88 μM	n.d.
% of hydrolysis	di-alanine	60%	n.d.
	hexa-alanine	n.d.	n.d.
concentration	glutamic acid	0.78 - 0.83 μM	1.5 - 2.5 μM
	di-glutamic acid	3.8 - 8.7 μM	1.9 - 4.0 μM
% of hydrolysis	di-glutamic acid	70%	n.d.

n.d. = not detectable

environmental conditions (Ehrlich 1990). Thus, oxic and anoxic conditions are certainly not the only influences on enzymatic activity.

Keil and Kirchman (1993) investigated protein utilization by heterotrophic bacteria in a Delaware estuary and coastal water. They found that assimilation of dissolved combined amino acids (DCAA) was inhibited by dissolved free amino acids (DFAA) when DFAA $\geq$ 0.050 μM. However, the concentrations of amino acids, peptides and hydrolyzable amino acids (Table 4.3) in SB and RB pore water do not have any consistent relationship to hydrolysis rates in sediment (Table 4.8).

One hypothesis for explaining the different rates of ^{3}H-peptide hydrolysis in SB and RB sediments is that it is related to different composition or concentration of sedimentary OM. SB has ten times more TOC than RB, and it is possible that there were more peptides (e.g., peptides containing alanine and lysine) on particle surfaces of SB which might compete with ^{3}H-peptides for enzyme binding sites. An alternative hypothesis is that different enzymes (because of the different microbial communities) are responsible for hydrolysis of ^{3}H-peptides in different sediments. A third idea is that the molecular form of peptides differs. Different pigment-protein complexes have been found which have different reactivates to enzymatic degradation because of variations in size, solubility, and molecular association (Larkum and Barrett 1983). Sun *et al.* (1993) reported that ^{14}C-Chl *a* was degraded at a rate and via a pathway different from Chl *a* naturally present in the sediment, due to a difference in MW. He reported that degradation pathways of chloropigments were likely dependent on the relative proportion of unassociated Chl *a* to chlorophyll complexes present in the sediment. In SB and RB, it is possible that sedimentary OM did not contain peptides with exactly the same structure as the added ^{3}H-peptides.

Table 4.8. Amino Acid and Peptide Concentrations in Pore Water vs. Peptide Hydrolysis Rate Constants in Sediments[1]

	amino acid or peptide	Skan Bay	Resurrection Bay
C_{aa}[2]	alanine	0.39 - 0.46	0.3 - 2.7
C_p[2]	di-alanine	n.d.	0.31 - 1.13 μM
	tri-alanine	n.d.- 0.615 μM	
	hexa-alanine	n.d. - 2.45 μM	4.25 - 10.46 μM
k_P[3]	di-alanine	0.30 h^{-1}	1.20 h^{-1}
	di-alanine	0.10 h^{-1}	0.20 h^{-1}
C_{aa}	lysine	0.06	n.d. - 0.2
C_p	di-lysine	0.13 - 1.79 μM	n.d. - 0.16 μM
k_P	di-lysine	0.07 h^{-1}	0.40 h^{-1}
C_{aa}	glutamic acid	0.13 - 0.31	0.2 - 8.0
C_p	di-glutamic acid	1.63 - 2.45 μM	1.54 - 1.6 μM
k_P	di-glutamic acid	1.0 h^{-1}	0.60 h^{-1}

[1] Fresh sediments.
[2] C_{aa} and C_p are amino acid and peptide concentrations in pore water from Table 4.2. n.d. = not detectable.
[3] k_P is the rate constant of peptide hydrolysis in sediments calculated using a tracer model.

Enzymes and electron acceptors for organic matter oxidation are different in aerobic and anaerobic environments (Ehrlich 1990). During degradation of ^{3}H-peptides, the enzymatic hydrolysis to amino acids was found to be the rate-limiting step under both oxic and anoxic conditions (Chapters 2 and 3), but the pathways of subsequent amino acid respiration are probably different. In oxic environments, the respiration to CO_2 and H_2O is carried out by single organism (Ehrlich 1990), while in anoxic environments, some of the respiration occurs via fermentation producing fatty acids, CO_2 and H_2O (Burdige 1991). However, if respiration is not the rate-limiting step for peptide degradation, the different pathways of amino acid catabolism should not control the total apparent degradation rates of peptides. Therefore, if there is no intrinsic difference in the enzymatic activity under oxic and anoxic conditions, there should not be any intrinsic difference in peptide degradation rates.

Comparison of decomposition rates of ^{3}H-peptides and other substances In the calculation of respiration rates of amino acids in SB and RB sediments, the rate constants calculated from the ^{3}H-tracer model and the amino acid concentrations from both previous studies and this work were used (Tables 2.3 and 3.2). The dissolved free amino acid concentrations in RB sediment pore waters measured in different studies varied greatly. But the order of amino acid mineralization rates was consistently glutamic acid > alanine > lysine, which is the same as the results for homogeneously ^{14}C-labeled amino acids obtained by Sugai and Henrichs (1992). The amino acid respiration rates in SB had a similar pattern, and their values were similar to those in RB, except that the lysine decomposition rate in SB was lower.

Both the concentrations of added ^{3}H-peptides and total peptides were used to calculate the hydrolytic rates (Tables 2.3 and 3.2), because the real peptide concentrations should be between the added concentration and the total concentration estimated by

HPLC. The total peptides were the peptide-like materials which were eluted from the HPLC at the same time as added ^{3}H-peptides. The minimum rates shown are those calculated using the added ^{3}H-peptide concentration and the maximum rates are those using the HPLC-measured concentration. The rates in SB were close to those in RB.

In anoxic SB, the total respiration rate (using the minimum value of amino acid concentration because most of the measurements obtained low values) of alanine, glutamic acid and lysine is 47 mol C m^{-2} yr^{-1}, which is similar to the acetate oxidation rates (52 mol C m^{-2} yr^{-1}) measured using ^{14}C-radiolabeled acetate (Shaw *et al.* 1984). Since fatty acids (including acetate) are the intermediate products of amino acid fermentation (Burdige 1991), the acetate oxidation rates are expected to be similar to those of amino acids. However, the total oxidation rate of the three amino acids is greater than the carbon-equivalent sulfate reduction rate measured by the $^{35}SO_4^{2-}$ tracer technique (18±1, Alperin *et al.* 1992). In anoxic sediments, amino acid oxidation rates should be much less than twice the sulfate reduction rate, since sulfate is the major terminal electron acceptor in the sediments (Henrichs 1993). However, acetate oxidation rates significantly greater than the carbon oxidation rates calculated from direct sulfate reduction rate measurements have been also reported by others (Christensen and Blackburn 1982; Parkes *et al.* 1984).

The total of the hydrolytic rates of ^{3}H-peptides (2.49 mol C m^{-2} yr^{-1} for ala_2, 2.44 for ala_6, 2.49 for lys_2, and 4.75 for glu_2) calculated from the added ^{3}H-peptide concentrations in SB sediment is less than the carbon-equivalent sulfate reduction rate, 18±1 (Alperin 1988; Alperin *et al.* 1992). Also, the total hydrolytic rate is much less than the oxidation rates of amino acids and acetate. This result supports the hypothesis that enzymatic hydrolysis is the rate-limiting step of peptide decomposition.

Preservation of Peptides in Sediments

The loss of peptides in sediments is either due to hydrolysis and then respiration by microorganisms or to adsorption onto sediment surfaces. Comparison of untreated and autoclaved sediments showed that rates of hydrolysis were generally greater than rates of adsorption. However, once the peptides were adsorbed, the hydrolytic rate of the adsorbed peptides (k_{PAE}) became less than that of the dissolved peptides (k_P) (Table 4.9). In both sediments, k_P/k_{PAE} ratios of lys_2 were greater than those of the other peptides, indicating the hydrolysis of the adsorbed alanyl and glutamyl peptides is slowed more by adsorption than that of lys_2. Adsorption still affects lys_2 hydrolysis more, however, because of the greater lys_2 adsorption. The adsorption and respiration of three amino acids in these two sediments has a very similar pattern to that of the peptides (Tables 2.2 and 3.1). This is consistent with the observation that neutral amino acids (e.g., glycine, and alanine) and acidic amino acids (e.g., glutamic acid and β-glutamic acid) are the major dissolved amino acid constituents of pore water while basic amino acids (e.g., lysine) are minor (e.g., Henrichs 1980; Henrichs *et al.* 1984; Henrichs and Farrington 1987). However, there probably are other reasons for this observation, such as the composition of microorganisms and the composition of hydrolyzable amino acids in the sediment.

Studies of clays and sediments have shown that adsorption of organic compounds decreases their biological availability (e.g., Dashman 1977; Marshman and Marshall 1981; Gordon and Millero 1985; Lorenz and Wackernagel 1987). How much adsorption inhibits organic compound degradation is dependent on how they are bound to the surface. For example, Samuelsson and Kirchman (1990) found that growth rates of attached bacteria on adsorbed algal protein were initially higher on hydrophilic glass than on hydrophobic polyethylene. Their hypothesis was that bond strengths of this protein to hydrophilic glass were greater, because of the stronger surface interactions and more steric protection, than

Table 4.9. Rate Constants of Peptide Hydrolysis in Two Marine Sediments

peptide	rate constant (h^{-1})*			
	k_P	k_{PAE}	k_P	k_{PAE}
	Skan Bay		Resurrection Bay	
di-lysine	0.007	0.01	0.40	0.01
di-glutamic acid	1.0	0.04	0.60	0.04
di-alanine	0.30	0.01	1.20	0.02
tri-alanine	0.20	0.02	n.d.	n.d.
hexa-alanine	0.10	0.02	0.20	0.02

* k_{PAE} is the hydrolysis rate constant of adsorbed peptide and k_P is the hydrolysis rate constant of dissolved peptide. Data are from Tables 2.2 and 3.1.
n.d. = not determined

bond strengths to hydrophobic polyethylene. It is expected that adsorption by covalent bonds should be more protective than that by ion exchange, and that conformation changes and creation of steric barriers during adsorption should also increase the protection of the adsorbed organic matter.

One hypothesized protection mechanism for sedimentary organic carbon is the formation of melanoidins (or humic substances), which are believed to be formed from protein and polysaccharide precursors (Hoering 1973; Hedges 1978; Ishiwatari *et al.* 1986; Rubinsztain *et al.* 1986; Yamanoto and Ishiwatari 1989). In a diagenetic experiment using carbonate skeletons, Collins *et al.* (1992) found that covalent linkages between amino acids of a protein (bovine serum albumin) and sugars were formed during *in vitro* melanoidin formation, and that these products had properties similar to those of organic matter in fossil biominerals. In marine sediments, Henrichs and Doyle (1986) reported that melanoidins synthesized from amino acids and glucose were decomposed on average 9 times more slowly than algae or a bacterial polymer. The irreversible adsorption of amino acids and peptides in marine sediments may in part be due to such condensation reactions (Henrichs and Sugai 1993; this study). However, the melanoidin formation or humification under *in situ* conditions may occur over years to hundreds of years' time, so that it is very difficult to obtain direct evidence for this process from experiments lasting hours or days.

An alternative hypothesis for a protection mechanism is that organic matter is protected by its location inside pores too small to allow functioning of the hydrolytic enzymes necessary for organic matter decay (Mayer 1994). Protein and peptides require enzymatic hydrolysis before they can be assimilated by organisms. Most hydrolytic enzymes require intimate contact between enzyme and substrate (Metzler 1977). Small pores could protect in two ways: first, by excluding contact between enzymes and organic

matter, and second, by favoring condensation reactions by binding reactive functional groups into close proximity within small pores. This pore protection could help to explain the relationship between organic carbon and grain size, found in most continental shelf sediments, in terms of the surface area of the sediments (Mayer 1994).

However, this hypothesis does not explain the preservation of organic matter in SB sediments completely. According to measurements by Mayer (1994), at a sediment depth of 2 - 4 cm (from which most samples used in this study were collected), the sedimentary organic carbon concentration is 5.5 mg-OC m^{-2}, which is much greater than the equivalent concentration (0.86 mg-OC m^{-2}) of an OM monolayer coating all mineral surfaces. Thus, the sedimentary OM in SB is much greater than the available pore volume of minerals. Moreover, most sedimentary OM is large enough (MW > 10 kD, Hayase and Tsubota 1983) to be excluded from small pores. Minerals in SB sediments are not necessarily the only source of small pores. It is possible that pores or spaces in sedimentary OM could protect peptides by a mechanism similar to that for mineral surfaces.

The preservation of total hydrolyzable amino acids (THAA) is strongly related to the preservation of organic matter, because THAA are major constituents of sediment organic matter in marine sediments. They make up 20 - 70% of the total nitrogen in most coastal sediments (Kvenvolden 1975; Schroeder and Bada 1976). In the upper meter of nearshore sediments, the THAA content and ratios of THAA nitrogen:TN decrease with depth, but usually by less than a factor of five (Henrichs and Farrington 1979; Henrichs 1980; Lee and Cronin 1982). THAA in marine sediments originally came from marine organisms. However, it is very difficult to trace directly the THAA content and composition back to the amino acid composition of the sources, because THAA composition varies little among different organisms (Henrichs 1980). The THAA

individual amino acid residue composition is remarkably uniform, both with depth in core and among varied sedimentary environments (Henrichs *et al.* 1984). Generally, glycine is the most abundant amino acid residue, with aspartic acid, alanine, and serine next.

One hypothesis for the formation of THAA in sediments is by the irreversible adsorption of amino acids (Henrichs and Sugai 1993). Basic amino acids (e.g., lysine and arginine) are adsorbed much more by marine sediments than acidic and neutral amino acids (e.g., glutamic acid, aspartic acid, and alanine) (e.g., Henrichs and Sugai 1993; Sugai and Henrichs, in preparation). The mineralization rate of lysine in sediments was found much slower than those of acidic and neutral amino acids (Sugai and Henrichs 1992). Thus, if adsorbed DFAAs were a major contributor to THAA, basic amino acids should predominate in THAA and basic amino acids should accumulate in THAA with depth. But this is found only to a very limited degree in marine sediments (Burdige and Marten 1988, 1991).

Although basic lys_2 is adsorbed by marine sediments more than acidic and neutral peptides, the difference in adsorption among different peptides is much smaller than that among different amino acids. This indicates that the adsorption of peptides becomes less selective based on composition with increasing MW. So, if adsorbed peptides contribute to the THAA in sediments, the basic amino acids will not necessarily be markedly enriched. The irreversible adsorption of dissolved combined amino acids by sediments can explain THAA composition and its changes with sediment depth better than DFAA adsorption alone.

Conclusions

Figure 4.2 shows how dissolved peptides are removed from sediment pore water:

1. One substantial sink of peptides is by hydrolysis which produces smaller

peptides and amino acids. The hydrolysis of peptides is related more to their molecular structures than to the redox conditions. There is no evidence that peptides are decomposed faster in oxic sediment than in anoxic sediment.

2. The adsorption of peptides, in terms of the partition coefficient K_{ads}, is much greater in SB sediment than in RB sediment. The differences in organic content (or composition) are mainly responsible for differences in adsorption between the two sediments. No clear effects of pore water composition or redox conditions on peptide adsorption were found. Basicity of peptides is an important factor in controlling adsorption, but hydrophobicity is not.

3. Although adsorption is small compared to enzymatic hydrolysis, adsorption decreases the decomposition rates of peptides in both oxic and anoxic sediments. Adsorption could be an important intermediate step leading to condensation reactions and the long-term preservation of peptides.

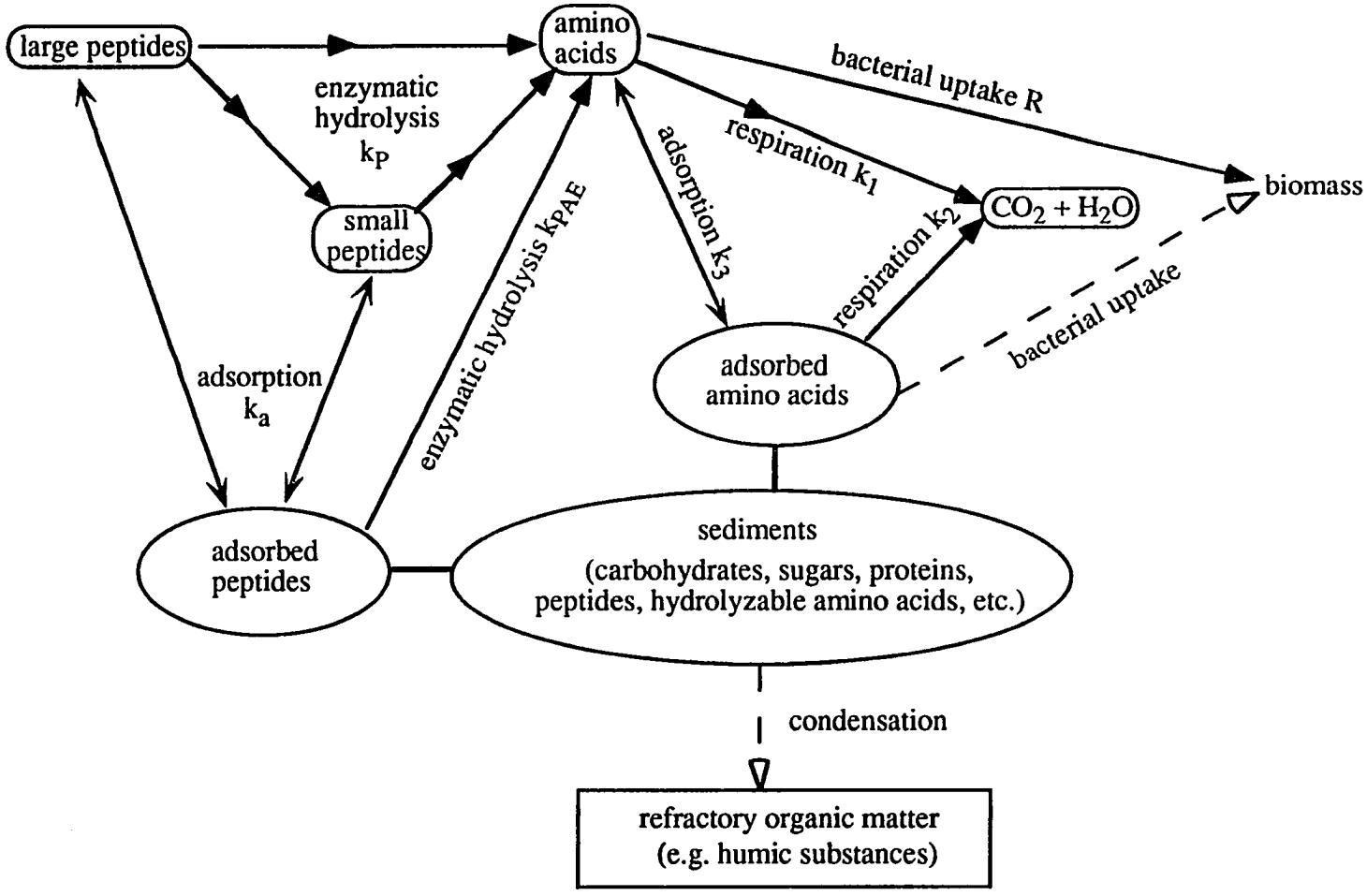


Figure 4.2. Fate of Peptides in Marine Sediments. k_P, k_a, k_{PAE}, k_1, k_2, k_3 and R are parameters from the tracer model (Tables 2.3 and 3.1). Major reactions are marked by double arrowheads in one direction, reversible reactions are marked by arrowheads in two directions, and reactions not included in model calculations are marked by dashed lines.

Chapter 5: Summary and Conclusions

In marine sediments, hydrolyzable amino acids are major constituents of the organic matter and their decomposition rates can be as slow as those of total organic carbon (Henrichs *et al.* 1984; Henrichs and Farrington 1987; Burdige and Martens 1988). However, the reasons why these compounds escape bacterial decomposition are unknown (Henrichs and Reeburgh 1987). Much previous research has focused on the decomposition and adsorption of free amino acids in marine sediments and in minerals. Although there are some studies on peptides and proteins, they were conducted using either pure minerals or artificial surfaces, not natural sediment particles and microbial communities. There have also been a few studies of enzymatic activity in marine sediments, but peptide analogs (dye-labeled substrates), rather than actual peptides, were usually used. Such substances may differ from true peptides in their metabolism by bacteria and, especially, in their adsorption by sediment.

In order to improve understanding of the processes leading to preservation of hydrolyzable amino acids, the decomposition and adsorption of peptides were investigated in two marine sediments from Alaska. Acidic di-glutamic acid (glu_2), basic di-lysine (lys_2), and neutral di-alanine (ala_2), tri-alanine (ala_3) and hexa-alanine (ala_6) were chosen to study how molecular structure influences decomposition and adsorption. The decomposition experiments conducted in Resurrection Bay (RB) and Skan Bay (SB) sediments showed how peptides were degraded under oxic and anoxic conditions. Peptides in pore water and sediments were hydrolyzed by enzymes to release amino acids, which were respired (or oxidized) to CO_2 and H_2O.

Little hydrolysis of peptides occurred in filtered pore water, except that in SB pore water, ala_2 and glu_2 were substantially hydrolyzed within 18 minutes. However, the hydrolysis in pore water ceased after 1 hour while some peptides still remained. This

nonlinear variation of hydrolysis with time indicates that the remaining peptide was not available to hydrolytic enzymes. The hydrolysis of ala_2 increased with ala_2 concentration, fitting the Lineweaver-Burk transform of the Michaelis-Menten equation. Similarly, the hydrolysis of glu_2 increased with glu_2 concentration, but the hydrolysis rate did not reach saturation even at the maximum solubility of glu_2 in pore water. Although the hydrolysis of these two peptides was rapid, the respiration of their hydrolytic products (alanine and glutamic acid) was very slow.

In sediments, the initial enzymatic hydrolysis rate of all peptides was greater than in pore water. Both hydrolysis and respiration increased with time and peptide concentrations. A tracer model was applied to calculate the rate constants, by fitting the model equations to the variation of hydrolysis and respiration with time. In this model, hydrolysis and adsorption of peptides and respiration and adsorption of the amino acids released by hydrolysis were considered. A diffusion model was also applied to calculate the rate constants, based on the variation of hydrolysis and respiration with peptide concentrations. Rates calculated using the tracer model were consistent with those calculated using the diffusion model. Under both oxic and anoxic conditions, the hydrolytic rate constants of alanyl and glutamyl peptides were greater than those of lysyl peptides, and the rate constants of alanyl peptides decreased with increasing molecular weight (or chain length). The hydrolysis rates of peptides were less than or equal to the respiration rates of the amino acids, indicating that hydrolysis was the rate-limiting step of peptide decomposition.

The concentrations of amino acids and peptides in pore water were not the major factors affecting the decomposition rates of the added ^{3}H-peptides. Nor was there a consistently greater rate of ^{3}H-peptide hydrolysis or ^{3}H-amino acid oxidation under the

oxic conditions in RB than under the anoxic conditions in SB. Different peptides were hydrolyzed at different rates, largely dependent on their molecular structures.

The enzymatic activity was very difficult to eliminate from sediments. One example is that the hydrolysis of peptides in stored sediments for two years was greater than in fresh sediments. Another example is that enzymatic activity could not be eliminated by freezing, formaldehyde, heating and autoclaving for up to 1 hour. Only autoclaving for 2 hours nearly eliminated hydrolysis.

Autoclaved sediments were used to study peptide adsorption. Autoclaving did not affect adsorption, because adsorption in autoclaved sediments was not statistically different from that in untreated sediments. Adsorption was a rapid process and reached apparent equilibrium in 1 to 5 hours. Over the peptide concentration range of 0.01 to 380 μM, adsorption increased with concentration and saturation of adsorption sites was not observed in either sediment. The adsorption vs. concentration relationship could be fitted by the Freundlich equation. The adsorption isotherms were nearly linear with concentration for all the peptides, suggesting that there were a large number of nearly equivalent adsorption sites on the sediment surface. In general, adsorption increased with basicity of the molecules; partition coefficients of lysyl peptides were greater than those of alanyl and glutamyl peptides. In SB sediment, adsorption decreased with increasing numbers of -COOH groups and molecular weight: $lys_2 > ala_2 > glu_2 > ala_3 > ala_6$. In contrast, the adsorption increased with increasing molecular weight in RB sediment: $ala_6 > lys_2 > ala_3 > ala_2 > glu_2$. Adsorption partition coefficients of peptides, especially of di-peptides, were much greater in SB than in RB sediments. Pretreatment experiments showed that 40 to 50% of adsorption was by cation exchange, probably between amino groups of peptides and $-COO^-$ groups of sedimentary organic matter.

However, some of the peptide adsorption was irreversible by ion-exchange solutions, e.g., 1 M cesium (chloride), 1 M (sodium) acetate, and seawater. The adsorbed peptides could not be completely extracted even by 0.2 N acid (HCl), and the acid extractability decreased with increasing incubation time, which indicated that some other reactions (e.g., condensation) may occur gradually with time.

Adsorption of small peptides (e.g., di- and tri-alanyl peptides) was similar to that of free amino acids. Basicity of the molecules played a more important role than molecular weight in enhancing peptide adsorption. Sedimentary organic matter is apparently the major adsorber for peptides. The higher total organic carbon content in SB than in RB was probably responsible for the greater adsorption of peptides in SB than in RB, as well as the different adsorption patterns in the two sediments. The peptide and amino acid concentrations in pore water did not influence the adsorption significantly.

Enzymatic hydrolysis and adsorption were the major processes removing peptides from pore waters. Hydrolysis was the major sink because the hydrolytic rate was greater than the adsorption rate. However, once peptides were adsorbed, their hydrolysis and respiration were greatly decreased compared to those of the dissolved peptides. Thus, adsorption could be an important step in the process of peptide preservation in sediments.

There are two hypothesized mechanisms for the preservation of peptides by adsorption. One is condensation reactions among organic compounds (e.g., proteins, peptides, amino acids, sugars) in sediments, which form refractory organic material. Another hypothesis is preservation in sediment pores. Mayer (1994) has published evidence that small mineral particles, which have a large surface area, strongly adsorb some organic molecules; but exclude enzymes because they are larger than the pores. This process could protect peptides from enzymatic hydrolysis. Moreover, the concentrated organic matter inside small pores could energetically favor organic matter condensation

reactions. However, this mechanism is probably not an important factor in Skan Bay sediments, where the organic content is much greater than could be accommodated in pores (Mayer 1994).

It has been the controversial whether anaerobic OM degradation rates are intrinsically lower than aerobic rates (Calvert and Pedersen 1992). However, hydrolysis and respiration rates of peptides in Skan Bay and Resurrection Bay sediments were quite similar, despite large differences in the electron-acceptors available for organic matter oxidation, sediment composition, and organic content. Although adsorption also affected the decomposition rates of both peptides and amino acids, adsorption effects were similar in the two sediments, because the greater porosity of Skan Bay sediment compensated for its greater adsorption per gram of particles. An interpretation consistent with the data is: Hydrolysis is the rate-limiting step in the mineralization of peptides in sediments. Although metabolic pathways of the released amino acids differ in oxic, suboxic, and anoxic sediments, this has little effect on mineralization or preservation. The important factor determining the rate of mineralization is the rate of hydrolysis, which in turn depends on hydrolytic enzyme activity, the structure of the molecules to be hydrolyzed, and whether these molecules are inaccessible to enzymes, due to adsorption.

References

Abelson, P. H., Hare, P. E. (1971). Reaction of amino acids with natural and artificial humus and kerogens. *Carnegie Inst. Wash. Yearb.* 69: 327-334.

Abrahamsson, K., Klick, S. (1991). Degradation of halogenated phenols in anoxic natural marine sediments. *Mar. Pollution Bulletin* 20(5): 227-233.

Aller, R. C. (1982). The effects of macrobenthos on chemical properties of marine sediment and overlying water. In: McCall, P. L., Trevesz, M. J. S. (ed.) Animal Sediment Relations. New York: Plenum, p. 53-102.

Aller, R. C., Yingst, J. Y. (1985). Effects of the marine deposit-feeders *Heteromastus filiformis* (polychaeta), *Macoma balthica* (bivalvia), and *Tellina texana* (bivalvia) on averaged sedimentary solute transport, reaction rates, and microbial distributions. *J. Mar. Res.* 43: 615-645.

Alperin, M. J. (1988). The carbon cycle in an anoxic marine sediment: concentrations, rates, isotope ratios, and diagenetic models. Ph.D. thesis, University of Alaska Fairbanks.

Alperin, M. J., Reeburgh, W. S. (1984). Geochemical observations supporting anaerobic methane oxidation. In: Crawford, R. L., Hanson, R. S. (ed.) Microbial Growth on C1 Compounds. American Society for Microbiology, Washington, D. C., p. 282-298.

Alperin, M. J., Reeburgh, W. S. (1985). Inhibition experiments on anaerobic methane oxidation. *Appl. Environ. Microbiol.* 50: 940-945.

Alperin, M. J., Reeburgh, W. S., Devol, A. H. (1992). Organic carbon remineralization and preservation in sediments of Skan Bay, Alaska. In: Whelan, J., Farrington, J. W. (ed.) Organic Matter: Productivity, Accumulation, and Preservation in Recent and Ancient Sediments. Columbia University Press, New York, p. 99-122.

Althel, C. B. (1979). Fundamentals of Enzyme Kinetics. Butterworth & Co. Ltd.

Andrade, J. D., Hlady, V. L., Van Wagenen, R. A. (1984). Effects of plasma protein adsorption on protein conformation and activity. *Pure Appl. Chem.* 56: 1345.

Ansbaek, K., Blackburn, T. H. (1980). A method for the analysis of acetate turnover in a coastal marine sediment. *Microb. Ecol.* 5: 235-264.

Berner, R. A. (1980). Early Diagenesis: A Theoretical Approach. Princeton University Press, Princeton, NJ, p. 241.

Berner, R. A. (1989). Biogeochemical cycles of carbon and sulfur and their effect on atmospheric oxygen over Phanerozoic time. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* (Global and planetary change section). 75: 97-122.

Billen, G. (1982). Modeling the processes of organic matter degradation and nutrient recycling in sedimentary system. In: Nedwell, D. B., Brown, C. M. (ed.) Sediment Microbiology. Academic Press, London, p. 15-52.

Bodanszky, M., Bodanszky, A. (1984a). The Practice of Peptide Synthesis. Springer-Verlag.

Bodanszky, M., Bodanszky, A. (1984b). Principles of Peptide Synthesis. Springer-Verlag.

Brownawell, B. J., Farrington, J. W. (1986). Biogeochemistry of PCB's in interstitial waters of a coastal marine sediment. *Geochim. Cosmochim. Acta* 50: 157-169.

Burdige, D. (1991). Microbial processes affecting alanine and glutamic acid in anoxic marine sediments. *FEMS Microb. Ecol.*. 85: 211-232.

Burdige, D. J., Martens, C. S. (1988). Biogeochemical cycling in an organic-rich coastal marine basin:10. The role of amino acids in sedimentary carbon and nitrogen cycling. *Geochim. Cosmochim. Acta* 52: 1571-1584.

Burdige, D. J., Martens, C. S. (1990). Biogeochemical cycling in an organic-rich coastal

marine basin:11. The sedimentary cycling of dissolved, free amino acids. *Geochim. Cosmochim. Acta* 54: 3033-3052.

Burns, R. G. (1978). Enzyme activity in soil: some theoretical and practical considerations. In: Burns, R. G. (ed.) Soil Enzymes. Academic Press, London, p. 295-340.

Calvert, S. E., Karlin, R. E., Toolin, L. J., Donahue, D. J., Southon, J. R., Vogel, J. S. (1991). Low organic carbon accumulation rates in Black Sea sediments. *Nature* 350: 692-695.

Calvert, S. E., Pedersen, T. F. (1992). Organic carbon in marine sediments: Is anoxia important? In: Whelan, J. K., Farrington, J. W. (ed.) Organic Matter: Productivity, Accumulation, and Preservation in Recent and Ancient Sediments. Columbia University Press, New York, p. 231-263.

Canfield, D. E. (1989). Sulfate reduction and oxic respiration in marine sediments: implications for organic carbon preservation in euxinic environments. *Deep-Sea Res.* 36: 121-138.

Carter, P. W. (1978). Adsorption of amino-acid-containing organic matter by calcite and quartz. *Geochim. Cosmochim. Acta* 42: 1239-1242.

Chanton, J. P., Martens, C. S., Goldhaber, M. B. (1987). Biogeochemical cycling in an organic-rich coastal marine basin: 7. Sulfur mass balance, oxygen uptake, and sulfide retention. *Geochim. Cosmochim. Acta* 51: 1187-1199.

Chen, Y., Schnitzer, M. (1978). The surface tension of aqueous solutions of soil humic substances. *Soil Sci.* 125: 7-15.

Christensen, D., Blackburn, T. H. (1980). Turnover of tracer (^{14}C, ^{3}H-labeled) alanine in inshore marine sediments. *Mar. Biol.* 58: 97-103.

Christensen, D., Blackburn, T. H. (1982). Turnover of ^{14}C-labeled acetate in marine

sediments. *Mar. Biol.* 71: 113-119.

Chróst, R. J. (1990). Microbial ectoenzymes in aquatic environments. In: Overbeck, J., Chróst, R. J. (ed.) Aquatic Microbial Ecology. Springer-Verlag, p. 47-78.

Cohn, E. J., Edsall, J. T. (1943). Protein, Amino Acids and Peptides as Ions and Dipolar Ions. Reinhold Publishing Corporation.

Collins, M. J., Westbroek, P., Muyzer, G., De Leeuw, J. W. (1992). Experimental evidence for condensation reaction between sugars and proteins in carbonate skeletons. *Geochim. Cosmochim. Acta* 56: 1539-1544.

Craven, D. B., Jahnke, R. A., Carlucci, A. F. (1986). Fine-scale distributions of microbial biomass and activity in California Borderland sediments. *Deep-Sea Research* 33: 379-390.

Crill, P. M., Martens, C. S. (1986). Methane production from bicarbonate and acetate in an anoxic marine sediment. *Geochim. Cosmochim. Acta* 50: 2089-2097.

Crill, P. M., Martens, C. S. (1987). Biogeochemical cycling in an organic-rich coastal marine basin. 6. Temporal and spatial variation in sulfate reduction rates. *Geochim. Cosmochim. Acta* 51: 1175-1186.

Dashman, T. (1977). Adsorption of amino acids and peptides on homoionic montmorillonite and kaolinite, and the utilization of the adsorbed amino acids and peptides by microorganisms. Ph.D. thesis, New York University.

Dashman, T., Stotzky, G. (1982). Adsorption and binding of peptides on homoionic montmorillonite and kaolinite. *Soil Biol. Biochem.* 14: 447-456.

Dashman, T., Stotzky, G. (1984). Adsorption and binding of peptides on homoionic montmorillonite and kaolinite. *Soil Biol. Biochem.* 16(1): 51-55.

Dashman, T., Stotzky, G. (1986). Microbial utilization of amino acids and a peptide bound on homoionic montmorillionite and kaolinite. *Soil Biol. Biochem.* 18: 5-14.

Davis, J. A. (1982). Adsorption of natural dissolved organic matter at the oxic-water interface. *Geochim. Cosmochim. Acta* 46: 2381-2393.

Davis, J., Gloor, R. (1981). Adsorption of dissolved organic matter in lake water by aluminum oxide: Effect of molecular weight. *Environ. Sci. Technol.* 15: 1223-1229.

Davis, J., Leckie, J. (1978). Effect of adsorbed complexing ligands on trace metal uptake by hydrous oxides. Environ. *Sci. Technol.* 12: 1309-1315.

Di Toro, D. M., Horzempa, L. M. (1982). Reversible and resistant components of PCB adsorption-desorption: Isotherms. *Environ. Sci. Technol.* 16: 594-602.

Di Toro, D. M., Manhony, J. D., Kirchgraber, P. R., O' Bryne, A. L., Pasquale, L. R., Piccirilli, D. C. (1986). Effects of nonreversibility, particle concentration, and ionic strength on heavy metal sorption. *Environ. Sci. Technol.* 20: 55-61.

Doyle, A. P. (1988). Respiration and adsorption of alanine, glutamic acid, lysine, and glucose in sediments from Resurrection Bay, Alaska. M.S. thesis, University of Alaska Fairbanks.

Ehrlich, H. L. (1990). Geomicrobiology. Marcel Dekker, Inc., p. 99-130.

Elwing, H., Welin, S., Askendal, A., Lundström, I. (1988). Adsorption of fibrinogen as measure of the distribution of methyl groups on silicon surfaces. *J. Colloid Interface Sci* . 123: 306.

Emerson, S. (1985). Organic carbon preservation in marine sediments. In: Sundquist, E., Broecker, W. S. (ed.) The Carbon Cycle and Atmospheric CO_2: Natural Variations Archaean to Present. Gegeophysical Monograph 32, American Geophysical Union, Washington, D.C., p. 78-87.

Emerson, S. E., Hedges, J. I. (1988). Processes controlling the organic carbon content of open ocean sediments. *Paleoceanogr.* 3: 621-634.

Engel, M. H., Macko, S. A. (1993). Organic Geochemistry: Principles and Applications.

Plenum Press, New York.

Farrington, J. W., Henrichs, S. M., Anderson, R. (1977). Fatty acids and Pb-210 geochronology of a sediment core from Buzzards Bay, Massachusetts. *Geochim. Cosmochim. Acta* 41: 289-296.

Fenchel, T., Blackburn, T. H. (1979). Bacteria and Mineral Cycling. Academic Press, London, p. 225.

Fenchel, T., Jørgensen, B. B. (1977). Detritus food chains of aquatic ecosystems: The role of bacteria. In: Alexander, M. (ed.) Advances in Microbial Ecology. Plenum Press, New York, p. 268.

Fernandez, V. L., Reimer, A. J., Denn, M. M. (1992). Magnetic resonance studies of polypeptides adsorbed on silica and hydroxyapatite surfaces. *J. Am. Chem. Soc.* 114: 9634-9642.

Fewson, C. A. (1988). Biodegradation of xenobiotic and other persistent compounds: The cause of recalcitrance. *TIBTECH.* 6: 148-153.

Folk, R. L. (1975). Petrology of Sedimentary Rocks. Hemphill Publishing Co.

Foree, E. G., McCarty, P. L. (1970). Anaerobic decomposition of algae. *Environ. Sci. Technol.* 4: 842-849.

Froelich, P. N., Klinkhammer, G. P., Bemder, M. L., Leudtke, N. A., Heath, G. R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B. (1979). Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim. Cosmochim. Acta* 43: 1075-1090.

Gordon, A. S., Millero, F. J. (1985). Adsorption mediated decrease in the biodegradation rate of organic compounds. *Microb. Ecol.* 11: 289-298.

Greenland, D. J., Laby, R. H., Quirk, J. P. (1962). Adsorption of glycine and its di-, tri, and tetra-peptides by montmorillonite. *Trans. Farad. Soc.* 58: 829-841.

Greenland, D. J., Laby, R. H., Quirk, J. P. (1965a). Adsorption of amino acids and peptides by montmorillonite and illite. Part 1. Cation exchange and proton transfer. *Trans. Faraday Soc.* 61: 2013-2023.

Greenland, D. J., Laby, R. H., Quirk, J. P. (1965b). Adsorption of amino acids and peptides by montmorillonite and illite. Part 2. Physical adsorption. *Trans. Faraday Soc.* 61: 2024-2035.

Griffith, P. C., Fletcher, M. (1991). Hydrolysis of protein and model dipeptide substrates by attached and nonattached marine *Pseudomonas sp.* strain NCIMB 2021. *Appl. Environ. Microbiol.* 57: 2186-2191.

Güde, H. (1978). Model experiments on regulation of bacterial polysaccharide degradation in lakes. *Archiv für Hydrobiologie* (supplement) 55: 157-185.

Haddad, R. I. (1989). Sources and reactivity of organic matter accumulation in a rapidly depositing, coastal marine sediment, Ph.D. Thesis, University of North Carolina at Chapel Hill.

Hagstrom, A., Ammerman, J. W., Henrichs, S., Azam, F. (1984). Bacterioplankton growth in seawater: 2. Organic matter utilization during steady-state growth in seawater cultures. *Mar. Ecol. Prog. Ser.* 18: 41-48.

Hanson, R. B., Gardner, W. S. (1978). Uptake and metabolism of two amino acids by anaerobic microorganisms in four diverse salt marsh soils. *Mar. Biol.* 46: 101-107.

Hansen, L. S., Holmer, M., Blackburn, T. H. (1993). Mineralization of organic nitrogen and carbon (fish food) added to anoxic sediment microcosms: role of sulfate reduction. *Mar. Ecol. Prog. Ser.* 102: 199-204.

Harvey, H., Fallon, R., Patton, J. (1986). The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. *Geochim. Cosmochim. Acta* 50: 795-804.

Haska, G. (1981). Activity of bacteriolytic enzymes adsorbed to clays. *Microb. Ecol.* 7: 331-334.

Hayase, K., Tsubota, H. (1983). Sedimentary humic acid and fulvic acid as surface active substances. *Geochim. Cosmochim. Acta* 47: 947-952.

Hedges, J. I. (1977). The association of organic molecules with clay minerals in aqueous solutions. *Geochim. Cosmochim. Acta* 41: 1119-1123.

Hedges, J. I. (1978). The formation and clay mineral reaction of melanoidins. *Geochim. Cosmochim. Acta* 42: 69-76.

Hedges, J. I., Hare, P. E. (1987). Amino acid adsorption by clay minerals in distilled water. *Geochim. Cosmochim. Acta* 51: 255-259.

Henrichs, S. M. (1980). Biogeochemistry of dissolved free amino acids in marine sediments. Ph.D. thesis, Woods Hole Oceanography Institution - Massachusetts Institute of Technology Joint Program.

Henrichs, S. M. (1992). Early diagenesis of organic matter in marine sediments: progress and perplexity. *Mar. Chem.* 39: 119-149.

Henrichs, S. M. (1993). Early diagenesis of organic matter: The dynamics (rates) of cycling of organic compounds. In: Engel, M. H., Stephen, A. M. (ed.) Organic Geochemistry. Plenum Press, New York, p. 101-117.

Henrichs, S. M., Doyle, A. P. (1986). Decomposition of ^{14}C-labeled organic substances in marine sediments. *Limnol. Oceanogr.* 31: 765-778.

Henrichs, S. M., Farrington, J. W. (1979). Amino acids in interstitial water of marine sediments. *Nature* 279: 319-322.

Henrichs, S. M., Farrington, J. W. (1987). Early diagenesis of amino acids and organic matter in two coastal marine sediments. *Geochim. Cosmochim. Acta* 51: 1-15.

Henrichs, S. M., Reeburgh, W. S. (1987). Anaerobic mineralization of marine sediment

organic matter: rates and the role of anaerobic processes in the oceanic carbon economy. *Geomicrobiol. J.* 5: 191-237.

Henrichs, S. M., Sugai, S. F. (1993). Adsorption of amino acids by Resurrection Bay (Alaska) sediments: Functional group effects. *Geochim. Cosmochim. Acta* 57: 823-835.

Henrichs, S. M., Farrington, J. W., Lee, C. (1984). Peru upwelling region sediments near 15°S. 2. Dissolved free and total hydrolyzable amino acids. *Limnol. Oceanogr.* 29: 20-34.

Hoering, T. C. (1973). A comparison of melanoidin and humic acid. *Carnegie Inst. Wash. Yearb.* 72: 682-690.

Hollibaugh, J. T., Azam, F. (1983). Microbial degradation of dissolved proteins in seawater. *Limnol. Oceanogr.* 28: 1104-1116.

Horbett, T. A., Brash, J. L. (1987). Proteins at interfaces: Current issues and future prospects. In: Brash, J. L., Horbett, T. A. (ed.) Proteins at Interfaces: Physicochemical and Biochemical Studies. ACS Symp. Series 343. Am. Chem. Soc., Washington, D. C., p. 1.

Hunter, K. A. (1980). Microelectrophoretic properties of surface-active organic matter in coastal seawater. *Limnol. Oceanogr.* 25: 807-822.

Hunter, K. A., Liss, P. S. (1979). The surface charge of suspended particles in an estuary and coastal water. *Nature* 282: 823-825.

Ishiwatari, R., Morinaga, S., Yamamoto, S., Machihara, T., Rubinsztain, Y., Isoselis, P., Aizemshtat, Z., Ikan, R. (1986). A study of formation mechanism of sedimentary humic substances - I. Characterization of synthetic humic substances (melanoidins) by alkaline potassium permanganate oxidation. *Org. Geochem.* 9: 11-23.

Iskandarani, Z., Pietrzyk, D. J. (1981). Liquid chromatography separation of amino

acids, peptides, and derivatives on a porous polystyene-divinylbenzene copolymer. *Anal. Chem.* 53: 489-495.

Jewell, W. J., McCarty, P. L. (1971). Aerobic decomposition of algae. *Environ. Sci. Technol.* 5: 1023-1031.

Jones, B. N., Paabo, S., Stein, S. (1981). Amino acid analysis and enzymatic sequence determination by an improved *o*-phthaldialdehyde precolumn labeling procedure. *J. Liquid Chromatogr.* 4: 565-586.

Jönsson, U., Lundström, I., Rönnberg, I. (1987). Immunoglobulin G and secretory fibronectin adsorption to silica. *J. Colloid Interface Sci.* 117: 127.

Jørgensen, B. B. (1982). Mineralization of organic matter in the sea bed-the role of sulfate reduction. *Nature* 296: 634-645.

Keil, R. G., Kirchman, D. L. (1993). Dissolved combined amino acids: Chemical form and utilization by marine bacteria. *Limnol. Oceanogr.* 38(6): 1256-1270.

Kim, J., ZoBell, C. E. (1974). Occurrence and activities of cell-free enzymes in oceanic environments. In: Colwell, R. R., Morita, R. Y. (ed.) Effect of the ocean environment on microbial activities. University Park, p. 368-385.

King, G. M. (1986). Characterization of ß-glucosidase activity in intertidal marine sediments. *Appl. Environ. Microbiol.* 51: 373-380.

King, G., Klug, M. (1982). Glucose metabolism in sediments of a eutrophic lake: tracer analysis of uptake and product formation. *Appl. Environ. Microbiol.* 44: 1308-1317.

Kirchman, D. L., Henry, D. L., Dexter, S. C. (1989). Adsorption of proteins to surfaces in seawater. *Mar. Chem.* 27: 201-217.

Korczynski, M. S. (1981). Sterilization. In: Gerhardt, P., Murray, R. G. E, Costilow, R. N., Nester, E. W., Wood, W. A., King, N. R., Phillips, G. B. (ed.) Manual of Methods for General Bacteriology. American Society for Microbiology, Washington

D. C., p. 476-486.

Kristensen, E., Blackburn, T. H. (1987). The fate of organic carbon and nitrogen in experimental marine sediment systems: influence of bioturbation and anoxia. *J. Mar. Res.* 45: 231-257.

Krom, M. D., Sholkovitz, E. R. (1977). Nature and reactions of dissolved organic matter in the interstitial waters of marine sediments. *Geochim. Cosmochim. Acta* 41: 1565-1573.

Kvenvolden, K. A. (1975). Advances in the geochemistry of amino acids. *Annu. Rev. Earth Planet Sci.* 3: 183-212.

Ladd, J. N. (1978). Origin and range of enzymes in soil. In: Burns, R. G. (ed.) Soil Enzymes. Academic press, London, p. 51-96.

Larkum, A. M. D., Barrett, J. (1983). Light-harvesting processes in algae. *Adv. Bot. Res.* 10: 3-219.

Lee, C. (1992). Controls on organic carbon preservation: The use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems. *Geochim. Cosmochim. Acta* 56: 3323-3335.

Lee, C., Cronin, C. (1982). The vertical flux of particulate organic nitrogen in the sea: Decomposition of amino acids in the Peru upwelling area. *J. Mar. Res.* 40: 227-251.

Lindroth, P., Mopper, K. (1979). High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with *o*-phthaldialdehyde. *Anal. Chem.* 51(11): 1667-1674.

Little, J. E., Sjogren, R. E., Carson, G. R. (1979). Measurement of proteolysis in natural waters. *Appl. Environ. Microbiol.* 37: 900-908.

Loder, T. C., Liss, P. S. (1985). Control by organic coatings of the surface charge of estuarine suspended particles. *Limnol. Oceanogr.* 30: 418-421.

Lorenz, M. G., Wackernagel, W. (1987). Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. *Appl. Environ. Microbiol.* 53: 2948-2952.

Mant, C. T., Hodges, R. S. (1991). High performance liquid chromatography of peptides and proteins: separation, analysis, and conformation. CRC press.

Marshman, N. A., Marshall, K. C. (1981). Bacterial growth on proteins in the presence of clay minerals. *Soil Biol. Biochem.* 13: 127-134.

Martens, C. S., Haddad, P. I., Chanton, J. P. (1992). Organic matter accumulation, remineralization, and burial in an anoxic coastal sediment. In: Whelan, J., Farrington, J. W. (ed.) Organic Matter: Productivity, Accumulation, and Preservation in Recent and Ancient Sediments. Columbia University Press, New York, p.82-98.

Mayer, L. M. (1989). Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat. *Limnol. Oceanogr.* 34: 973-981.

Mayer, L. M. (1993). Organic matter at the sediment-water interface. In: Engel M. H., Stephen, A. M. (ed.) Organic geochemistry. Plenum Press, New York, p. 171-184.

Mayer, L. C. (1994). Surface area control of organic carbon accumulation in continental shelf sediments. *Geochim. Cosmochim. Acta* 58(4): 1271-1284.

Mayer, L. M., Rice, D. L. (1992). Early diagenesis of protein: A seasonal study. *Limnol. Oceanogr.* 37(2): 280-295.

Mayer, L. M., Rossi, P. M. (1982). Specific surface areas in coastal sediments: relationships with other textural factors. *Mar. Geol.* 45: 241-252.

McDaniel, T. B. (1989). Biological availability of amino acids in marine sediments. M.S. thesis, University of Alaska Fairbanks.

McGurie, J., Krisdhasima, V. (1991). Surface chemical influences on protein adsorption kinetics. *Food Tech.* p. 92 - 96.

Mendenhall, W. (1987). Introduction to probability and statistics. PWS-KENT.

Metzler, D. E. (1977). Biochemistry. Academic Press Inc., p. 315-350.

Meyer-Reil, L. A. (1983). Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight. II. Analysis of benthic bacterial populations. *Mar. Biol.* 77: 247-256.

Meyer-Reil, L. A. (1986). Measurement of hydrolytic activity and incorporation of dissolved organic substrates by microorganisms in marine sediments. *Mar. Ecol. Prog. Ser.* 31: 43-149.

Meyer-Reil, L. A. (1987). Seasonal and spatial distribution of extracellular enzymatic activities and microbial incorporation of dissolved organic substrates in marine sediments. *Appl. Environ. Microbiol.* 53(8): 1748-1755.

Meyer-Reil, L. A. (1990). Microorganisms in marine sediments: considerations concerning activity measurements. *Archiv für Hydrobuiologie Beihefte Ergebnisse Limnologie* 34: 1-6.

Meyer-Reil, L. A. (1991). Ecological aspects of enzymatic activity in marine sediments. In: Ryszard, J. C. (ed.) Microbial enzymes in aquatic environments. Springer-Verlag, p. 84-95.

Meyer-Reil, L. A., Bölter, M., Dawson, R., Liebezeit, G., Szwerinski, H., Wolter, K. (1980). Interrelationships between microbiological and chemical parameters of sandy beach sediments, a summer aspect. *Appl. Environ. Microbiol.* 39: 797-802.

Meyers, P. A., Quinn, J. G. (1973). Factors affecting the association of fatty acids with mineral particles in seawater. *Geochim. Cosmochim. Acta* 37: 1745-1759.

Müller, P. J., Suess, E. (1979). Productivity, sedimentation, and sedimentary organic carbon in the oceans. I. organic carbon preservation. *Deep-sea Res.* 26: 1347-1362.

Mycke, B., Michaelis, W., Degens, E. T. (1988). Biomarkers in sedimentary sulfides of Precambrian age. *Org. Geochem.* 13: 619-625.

Neihoff, R. A., Loeb, G. I. (1972). The surface charge of particulate matter in seawater. *Limnol. Oceanogr.* 17: 7-16.

Nimura, N., Suzuki, T., Kasahara, Y., Kinoshita, T. (1981). Reversed-phase liquid chromatographic resolution of amino acid enantiomers by mixed chelate complexation. *Anal. Chem* 53: 1380-1383.

Nissenbaum, A. (1974). The organic geochemistry of marine and terrestrial humic substances: implications of carbon and hydrogen isotope studies. In: Tissot, B., Bienner, F. (ed.) Organic Geochemistry. Editions Technip, Paris.

Novitsky, J. A. (1983). Microbial activity at the sediment-water interface in Halifax Harbor, Canada. *Appl. Environ. Microbiol.* 45: 1761-1766.

Ogram, A. V., Mathot, M. L., Harsh, J. B., Boyle, J., Pettigrew, C. A. Jr. (1993). Effect of DNA polymer length on its adsorption to soils. *Appl. Environ. Microbiol.* 60(2): 393-396.

Oshrain, R. L., Wiebe, W. J. (1979). Arylsulfatase activity in salt marsh soils. *Appl. Environ. Microbiol.* 38: 337-340.

Otsuki, A., Hanya, T. (1972a). Production of dissolved organic matter from dead algal cells. I. Aerobic microbial decomposition. *Limnol. Oceanogr.* 7: 248-257.

Otsuki, A., Hanya, T. (1972b). Production of dissolved organic matter from dead algal cells. II. Aerobic microbial decomposition. *Limnol. Oceanogr.* 7: 258-264.

Palacas, J. G., Swanson, V. E., Moore, G. W. (1966). Organic geochemistry of three North Pacific deep sea sediment samples. U. S. Geol. Surv. Prof. Pap. 550C, p. C102-C107.

Parkes, R. J., Taylor, J., Jorck-Ramberg, D. (1984). Demonstration, using *Desulfobacter* sp., of two pools of acetate with differing biological availability in marine pore water. *Mar. Biol.* 83: 271-276.

Premuzic, E. T., Benkovitz, C. M., Gaffney, J. S., Walsh, J. J. (1982). The nature and distribution of organic matter in the surface sediments of world oceans and seas. *Org. Geochem.* 4: 63-77.

Price, N. B. (1976). Chemical diagenesis in sediments. In: Riley, J. P., Chester, R. (ed.) Chemical Oceanography, 2nd edition. London: Academic Press, 6: 1-58.

Priest, F. G. (1984). Extracellular Enzymes. Van Nostrand Reinhold (UK) Co. Ltd., Workingham. p. 79.

Rashid, M. A. (1985). Geochemistry of Marine Humic Compounds. Springer-Verlag.

Raspor, B., Valenta, P. (1988). Adsorption of humic substances isolated from marine and estuarine sediments. *Mar. Chem.* 25: 211-226.

Reeburgh, W. S. (1980). Anaerobic methane oxidation: rate depth distributions in Skan Bay sediments. *Earth Planet. Sci. Lett.* 47: 345-352.

Reeburgh, W. S. (1983). Rates of biogeochemical processes in anoxic sediments. *Ann. Rev. Earth Planet. Sci.* 11: 269-294.

Reichardt, W. (1986). Enzymatic potential for decomposition of detritus biopolymers in sediments from Kiel Bay. *Ophelia.* 26: 369-384.

Rheinheimer, G. (1985). Aquatic Microbiology, 3th edition. John Wiley and Sons.

Robertson, B. R., Button, D. K. (1989). Characterizing aquatic bacteria according to population, cell size, and apparent DNA content by flow cytometry. *Cytometry* 10: 70-76.

Robertson, K. J., Williams, P. M., Bada, J. L. (1987). Acid hydrolysis of dissolved combined amino acids in seawater: a precautionary note. *Limnol. Oceanogr.* 32(4): 996-997.

Romanowski, G., Lorenz, M. G., Wackernagel, W. (1991). Adsorption of plasmid DNA to mineral surfaces and protection against DNAase I. *Appl. Environ. Microbiol.*

57(4): 1057-1061.

Rosenfeld, J. K. (1979). Amino acid diagenesis and adsorption in near shore anoxic sediments. *Limnol. Oceanogr.* 24: 1014-1021.

Rubinsztain, Y., Yariv, S., Aizenshtat, Z., Ikan, R. (1986). Characterization of melanoidins by IR spectroscopy II - I. Galactoseglycine melanoidins. *Org. Geochem.* 9: 117-125.

Samuelsson, M. A., Kirchman, D. L. (1990). Degradation of adsorbed protein by attached bacteria in relationship to surface hydrophobicity. *Appl. Environ. Microbiol.* 56: 3643-3648.

Sansone, F. J., Andrews, C. C., Okamoto, M. Y. (1987). Adsorption of short-chain organic acids onto nearshore marine sediments. *Geochim. Cosmochim. Acta* 51:1889-1896.

Sayler, G. S., Puziss, M., Silver, M. (1979). Alkaline phosphatase assay for freshwater sediments: application to perturbed sediment systems. *Appl. Environ. Microbiol.* 38: 922-927.

Schlesinger, W. H. (1991). Biogeochemistry: An Analysis of Global Change. Academic Press, Inc.

Schroeder, R. A., Bada, J. L. (1976). A review of the geochemical applications of the amino acid racemization reaction. *Earth Sci. Rev.* 12: 347-391.

Shaw, D. G., McIntosh, D. J. (1990). Acetate in recent anoxic sediments: Direct and indirect measurements of concentration and turnover rates. *Estuarine Coastal Shelf Sci.* 31: 775-788.

Shaw, D. G., Alperin, M. J., Reeburgh, W. S., MaCintosh, D. L. (1984). Biogeochemistry of acetate in anoxic sediments of Skan Bay, Alaska. *Geochim. Cosmochim. Acta.* 48: 1819-1825.

Sieskind, O., Wey, M. (1959). Sur l'adsorption d'acides amines par la montmorillonite-H. Influence de la position relative des deux fonctions -NH et -COOH. *Comptes Rendus Academie des Sciences, Paris.* 248: 1652-1655.

Sieskind, O., Wyart, J. (1960a). Etudes des complexes d'adsorption formes entre la montmorillonite-H et certain acides amines. Isotherms d'adsorption a pH 2 et a 20°C. *Compt. Rend.* 250: 2228-2230.

Sieskind, O., Wyart, J. (1960b) Sur les complexes d'adsorption formes en milieu acide entre la montmorillonite-H et certain acides amines: Leur structure. *Compt. Rend.* 250: 2392-2393.

Sigg, L., Stumm, W. (1981). The interaction of anions and weak acids with the hydrous goethite (α–FeOOH) surface. *Colloids Surf.* 2: 101-117.

Somville, M., Billen, G. (1983). A method for determining exoproteolytic activity in natural waters. *Limnol. Oceanogr.* 28: 190-193.

Stein, R. J., Rullkotter, J., Welte, D. H. (1986). Accumulation of organic-carbon-rich sediments in the late Jurassic and Cretaceous Atlantic Ocean-a synthesis. *Chem. Geol.* 56: 1-32.

Stewart, G. J., Sinigalliano, C. D., Garko, K. A. (1991). Binding of exogenous DNA to marine sediments and the effect of DNA/sediment binding on natural transformation of *Pseudomonas stutzeri* strain ZoBell in sediment columns. *FEMS Microbiol. Eco.* 85: 1-8.

Stumm, W. (1987). Aquatic Surface Chemistry. John Wiley & Sons.

Sugai, S. F., Henrichs, S. M. (1992). Rates of amino acid decomposition in Resurrection Bay (Alaska) sediments. *Mar. Ecol. Prog. Ser.* 88: 129-141.

Sugai, S. F., Henrichs, S. M. (in preparation). Adsorption and decomposition of amino acid in Skan Bay (Alaska) sediments.

Summons, R. E., Brassel, S. C., Eglinton, G., Evans, E., Horodyski, R. J., Robinson, N., Ward, D. (1988). Distinctive hydrocarbon biomarkers from fossiliferous sediment of the late Proterozoic Walcott Member, Chuar Group, Grand Canyon, Arizona. *Geochim. Cosmochim. Acta* 52: 2635-2657.

Sun, M.-Y., Lee, C., Aller, R. C. (1993). Anoxic and oxic degradation of ^{14}C-labeled chloropigments and a ^{14}C-labeled diatom in Long Island Sound sediments. *Limnol. Oceanogr.* 38(7): 1438-1451.

Talibudeen, O. (1955). Complex formation between montmorillonoid clays and amino acids and proteins. *Trans. Faraday Soc.* 51:582-590.

Taylor, G. T., Troy, P. J., Sharma, S. K. (1994). Protein adsorption from seawater onto solid substrata, I. Influences of substratum surface properties and protein concentration. *Mar. Chem.* 45: 15-30.

Tegelaar, E. W., de Leeuw, J. W., Derenne, S., Largeau, C. (1989). A reappraisal of kerogen formation. *Geochim. Cosmochim. Acta* 53: 3103-3106.

Theng, B. K. G. (1979). The Chemistry of Clay-Organic Reactions. John Wiley & Sons.

Tipping, E., Cooke, D. (1982). The effect of adsorbed humic substances on the surface charge of goethite (α-FeOOH) in freshwaters. *Geochim. Cosmochim. Acta* . 46: 75-80.

Tupas, L., Koike, I. (1990). Amino acid and ammonium utilization by heterotrophic marine bacteria grown in enriched seawater. *Limnol. Oceanogr.* 35: 1145-1155.

Wainwright, M. (1981). Assay and properties of alginate lyase and 1,3-ß-glucanase in intertidal sands. *Plant Soil* 59: 83-89.

Wang, X. C., Lee, C. (1990). The distribution and adsorption behavior of aliphatic amines in marine and lacustrine sediments. *Geochim. Cosmochim. Acta* 54: 2759-2774.

Wang, X. C., Lee, C. (1993). Adsorption and desorption of aliphatic amines, amino acids and acetate by clay minerals and marine sediments. *Mar. Chem.* 44: 1-23.

Weiss, A. (1969). Organic derivatives of clay minerals, zeolites, and related minerals. In: Engel, G., Murphy, M. T. J. (ed.) Organic geochemistry. Springer, New York, p. 737-781.

Westrich, J. T., Berner, R. A. (1984). The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested. *Limnol. Oceanogr.* 29: 236-249.

Williams, P. J. leB (1973). The validity of the application of simple kinetic analysis to heterogeneous microbial populations. *Limnol. Oceanogr.* 18: 159-165.

Wright, R. T., Burnison, B. K. (1979). Heterotrophic activity measured with radiolabeled organic substrates. In: Costerton, J. W., Colwell, R. R. (ed.) Native Aquatic Bacteria: Enumeration, Activity, and Ecology. American Society for Testing and Materials. ASTM STP 695, p. 140-155.

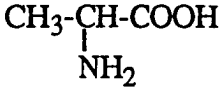
Wright, R. T., Hobbie, J. E. (1965). The uptake of organic solutes in lake water. *Limnol. Oceanogr.* 10: 22-28

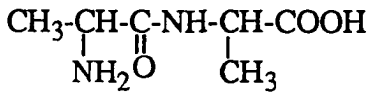
Wright, R. T., Hobbie, J. E. (1966). Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology* 47: 447-464.

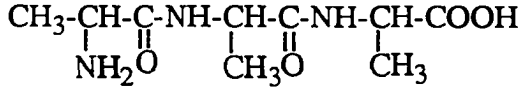
Yamanoto, S., Ishiwatari, R. (1989). A study of the formation mechanism of sedimentary humic substances - II. Protein based melanoidin model. *Org. Geochem.* 14: 479-489.

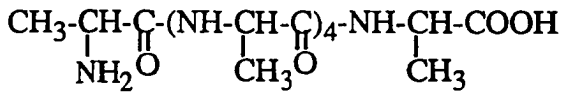
APPENDIX I: Amino Acid and Peptide Abbreviations

aaa α - aminoadipic acid

ala alanine 

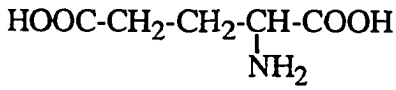
ala_2 alanylalanine 

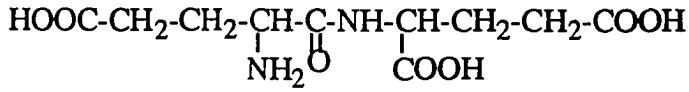
ala_3 alanylalanylalanine 

ala_6 alanylalanylalanylalanylalanylalanine 

DFAA dissolved free amino acid

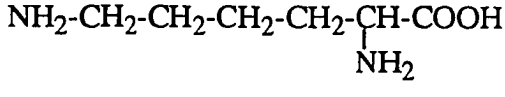
FAA free amino acid

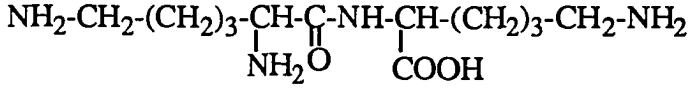
glu glutamic acid 

glu_2 glutamylglutamic acid 

gly glycine

leu leucine

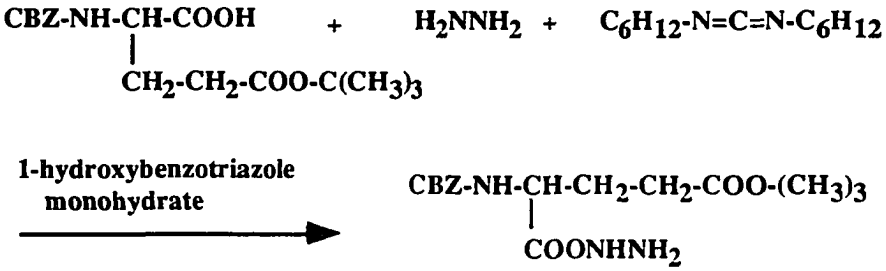
lys lysine 

lys_2 lysyllysine 

APPENDIX II: Procedures for ^{3}H-Peptide Synthesis and Purification

I. Synthesis of ^{3}H-glu$_2$ from N-CBZ-glu-γ-buty ester and ^{3}H-glu

1. Preparation of hydrazide



(1) Dissolve 16.87 mg (0.05 mmol) N-CBZ-glu-γ-buty ester in 130 μL N,N-dimethylformanide (DMF), cool in ice-water bath and stir vigorously.

(2) Flush the vial with N_2 while adding 3 μL (3 mg, 0.10 mmol) anhydrous hydrazine (H_2NNH_2), 17 mg (0.11 mmol) 1-hydroxybenzotriazole monohydrate, and finally 12 mg (0.06 mmol) dicyclohexylcarbodimide.

(3) Cap the vial while flushing with N_2 and wrap it with aluminum foil.

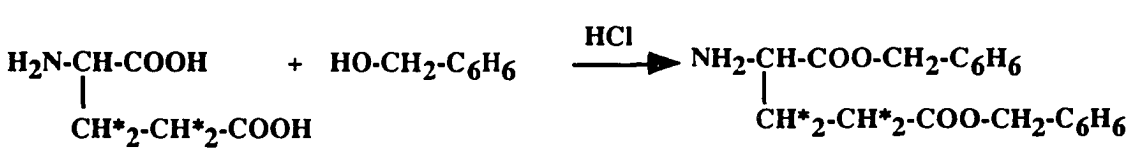
(4) Stir for 2 hours in ice bath and then at room temperature overnight.

(5) Centrifuge and remove the precipitate, evaporate DMF.

(6) Add 1 mL ethyl acetate, then wash with 0.5 mL water twice. If the hydrazide is insoluble in ethyl acetate, evaporate the ethyl acetate and triturate the residue with 0.5 mL water twice.

(7) Dry *in vacuo*.

2. Preparation of di-benzyl-^{3}H-glu ester



(1) Dry 240 μL ^{3}H-glu [3,4-^{3}H] (0.24 mCi, 0.01 mmol) in 0.01 N HCl solution *in vacuo*.

(2) Add 60 μL benzyl alcohol to dissolve the residue.

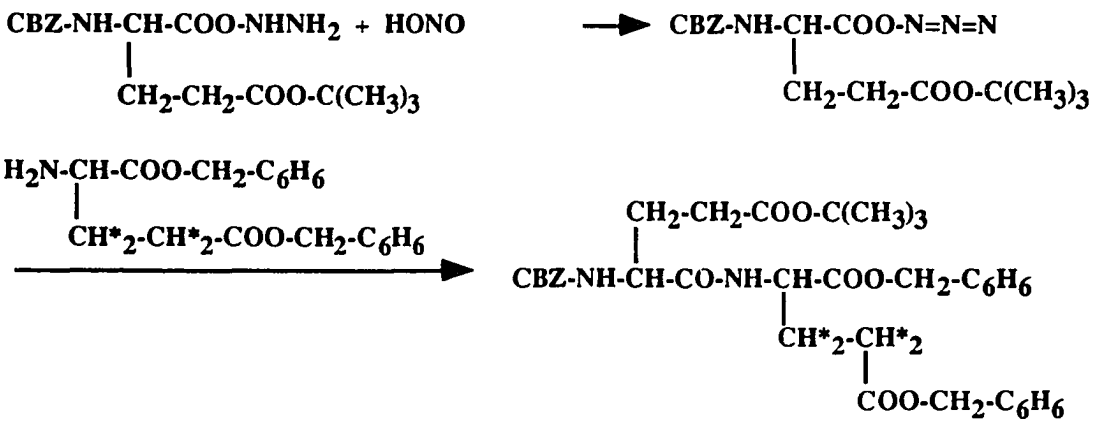
(3) Heat to 55°C in water bath, then bubble with dry HCl for 2 hours.

(4) Add 10 μL toluene, and remove the toluene *in vacuo* (About one-half h at 85-90°C is required).

(5) Bubble with dry HCl for 1 hour.

(6) Remove the solvent *in vacuo* at 85-90°C.

3. Conversion of the hydrazide to the azide and coupling of the benzyl ester with the azide



(1) Dissolve the glu-hydrazide from Step 1 (about 0.03 mmol) in a mixture of glacial acetic acid (36 μL), 5 N HCl (15 μL), and water (150 μL), stir and cool to - 5°C.

(2) Add $NaNO_2$ (2.8 mg, 0.031 mmol) in water (15 μL), and react for 10 minutes.

(3) Extract with 200 μL cold ether, discard the aqueous layer, wash the ether layer twice with ice water (120 μL).

OR (1) Dissolve the glu-hydrazide from Step 1 (about 0.03 mmol) in 300 μL DMF. Take a 120 μL aliquot, cool to - 5°C, and add 4 N HCl (18 μL) while stirring.

(2) Add 58 μL DMF and 5 N $NaNO_2$ in 4.5 μL water, stir for 5 minutes.

(3) Add 6.9 μL (0.05 mmol) triethylamine.

(4) Dry over anhydrous Na_2SO_4 briefly.

(5) Mix 1/40 of the volume of the solution (about 1.5 μmol of intermediate product) with ^{3}H-glu-benzyl ester in 20 μL chloroform (or DMF if insoluble).

(6) React at 10°C for 2.5 days.

(7) Remove solvent *in vacuo,* then dissolve residue in ethyl acetate, and wash sequentially with 50 μL of 0.5 N HCl, water, 0.5 N $KHCO_3$, and water.

(8) Remove solvent.

4. Deprotection of butyl ester (BOC) group

Add 68 μL trifluoroacetic acid and leave at room temperature for 2 hours, then dry *in vacuo*.

5. Deprotection of benzyl ester (CBZ) group by hydrogenation

(1) Dissolve residue from Step 4 in 1.5 μL methanol, and flush with nitrogen for 10 minutes.

(2) Add Pd-catalyst, flush with nitrogen again.

(3) Flush with hydrogen for 2 hours.

(4) Flush with nitrogen for 10 minutes.

(5) Filter out catalyst, remove methanol.

6. Purification by TLC

Develop the product from Step 5 on pre-coated TLC plates (silica Gel 60, silanized) with solution of acetone:water (4.5:1), $R_{glu} = 0.43$, $R_{glu_2} = 0.29$ where R = (distance that amino acid or peptide moved) (distance that solvent moved)$^{-1}$.

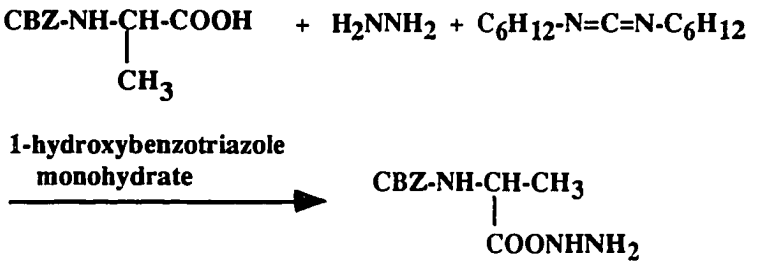
7. Purification by HPLC

Lichrosorb RP-18 (250 mm x 4.0 mm, 10 μM particle size) column was used. Elute with a solvent composition of 100% 0.1% acetic acid (adjust pH to 1.2-1.6 with 6 N HCl) for

5 minutes, then run a linear gradient to 100% methanol in 40 min. Flow rate was 1 mL/min. Collect the ^{3}H-glu$_2$-containing fraction of the eluate between 6 and 9 minutes.

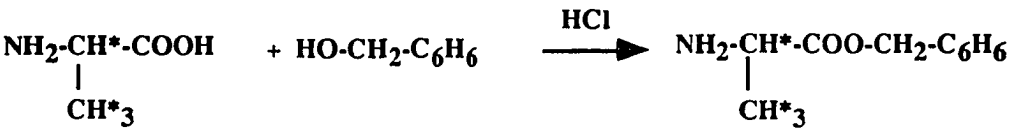
II. Synthesis of ^{3}H-ala$_2$ from N-CBZ-ala and ^{3}H-ala

1. Preparation of hydrazide



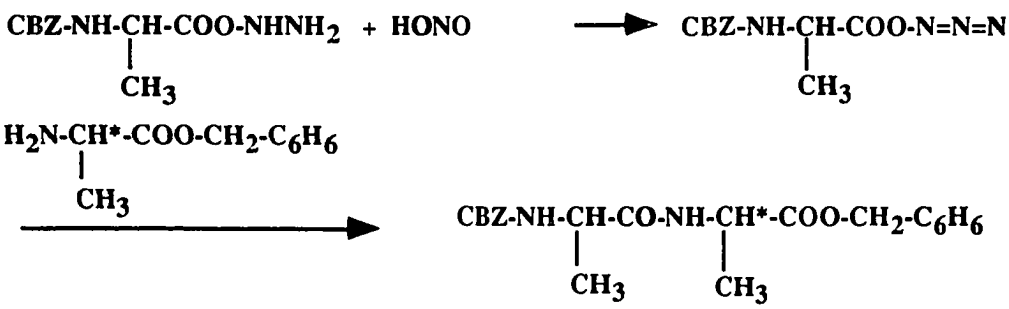
Procedure is similar to Step 1 of ^{3}H-glu$_2$ synthesis.

2. Preparation of ^{3}H-ala-benzyl ester



Procedure is similar to Step 2 of ^{3}H-glu$_2$ synthesis.

3. Conversion of the hydrazide to the azide and coupling of the benzyl ester with the azide



Procedure is similar to Step 3 of ^{3}H-glu$_2$ synthesis.

4. Deprotection of the benzyl ester (CBZ) group by hydrogenation

Procedure is similar to Step 5 of ^{3}H-glu$_2$ synthesis.

5. Purification by TLC

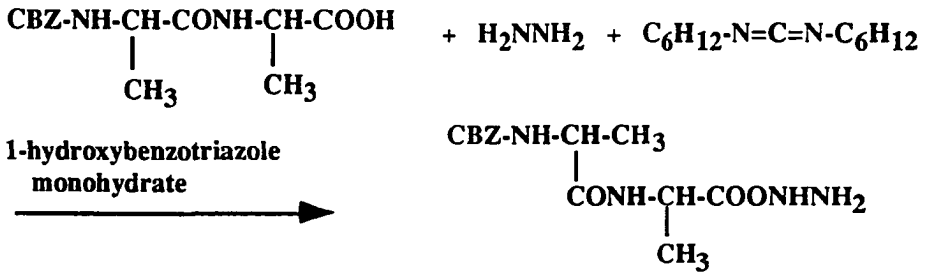
Develop the product from Step 4 on pre-coated TLC plates (silica Gel 60, silanized) with a solution of acetone:water (4:1), $R_{ala} = 0.29$, $Rala_2 = 0.19$.

6. Purification by HPLC

HPLC conditions are similar to Step 7 of ^3H-glu_2 synthesis. Collect the ^3H-ala_2-containing fraction of the eluate between 5 and 9 minutes.

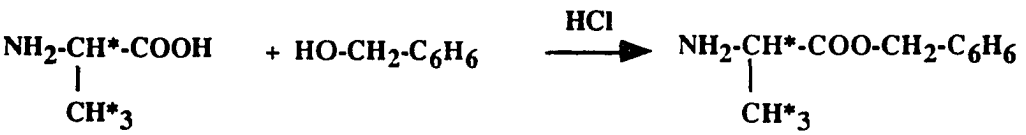
III. Synthesis of ^3H-ala_3 from N-CBZ-ala_2 and ^3H-ala

1. Preparation of hydrazide



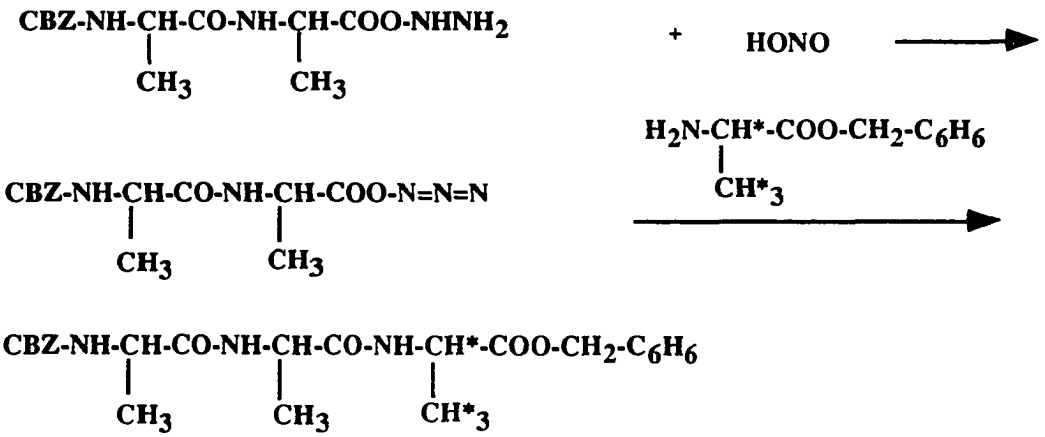
Procedure is similar to Step 1 of ^3H-glu_2 synthesis.

2. Preparation of ^3H-ala-benzyl ester



Procedure is similar to Step 2 of ^3H-glu_2 synthesis.

3. Conversion of the hydrazide to the azide and coupling of the benzyl ester with the azide



Procedure is similar to Step 3 of ^{3}H-glu$_2$ synthesis.

4. Deprotection of the benzyl ester (CBZ) group by hydrogenation

Procedure is similar to Step 5 of ^{3}H-glu$_2$ synthesis.

5. Purification by TLC

Develop the product from Step 4 on pre-coated TLC plates (silica Gel 60, silanized) with a solution of acetone:methanol:water (7:1:2), R_{ala} = 0.43, R_{ala_2} = 0.46, R_{ala_3} = 0.55.

6. Purification by HPLC

HPLC conditions are similar to Step 7 of ^{3}H-glu$_2$ synthesis. Collect the ^{3}H-ala$_3$-containing fraction of the eluate between 5 and 8 minutes.

IV. Synthesis of ^{3}H-ala$_6$ from ala$_5$ and ^{3}H-ala

1. Preparation of N-CBZ-ala$_5$

(1) Dissolve 10 mg (0.027 mmol) ala$_5$ in 200 μL 1 N NaOH. Heat if not dissolved at room temperature.

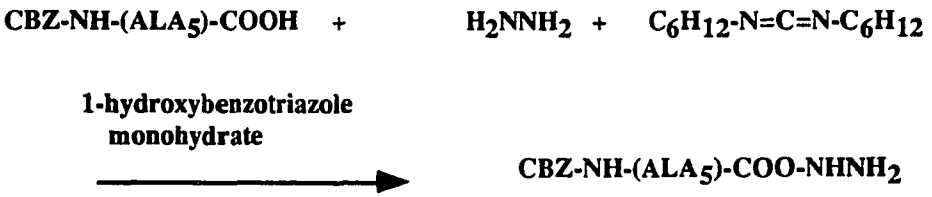
(2) Add benzyl chlorocarbonate (4.3 μL, 0.03 mmol), stir at room temperature for 3 hours.

(3) Extract with 400 μL of ether 4 times.

(4) Add 6 N HCl (130 μL).

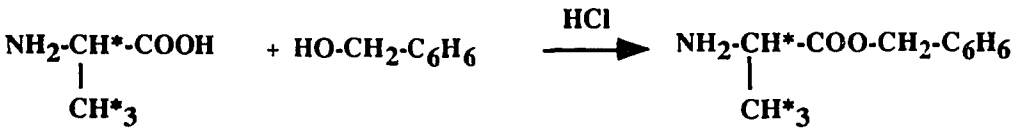
(5) Dry *in vacuo*.

2. Preparation of hydrazide



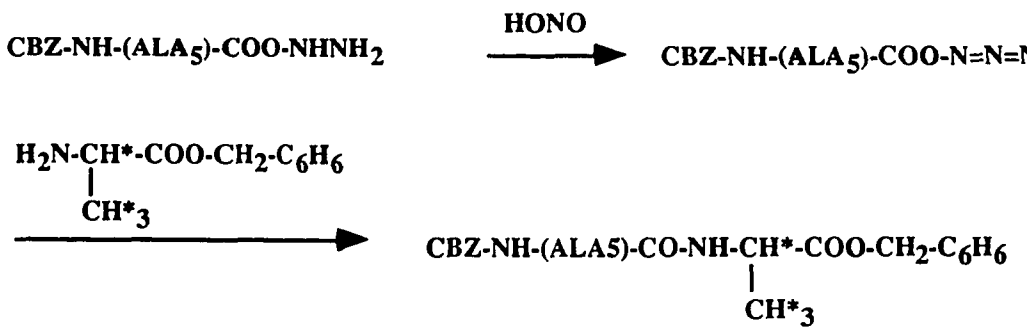
Procedure is similar to Step 1 of ^{3}H-glu$_2$ synthesis.

3. Preparation of ^{3}H-ala-benzyl ester



Procedure is similar to Step 2 of ^{3}H-glu$_2$ synthesis.

4. Conversion of the hydrazide to the azide and coupling of the benzyl ester with the azide



Procedure is similar to Step 3 of ^{3}H-glu$_2$ synthesis.

5. Deprotection of the benzyl ester (CBZ) group by hydrogenation

Procedure is similar to Step 5 of ^{3}H-glu$_2$ synthesis.

6. Purification by TLC

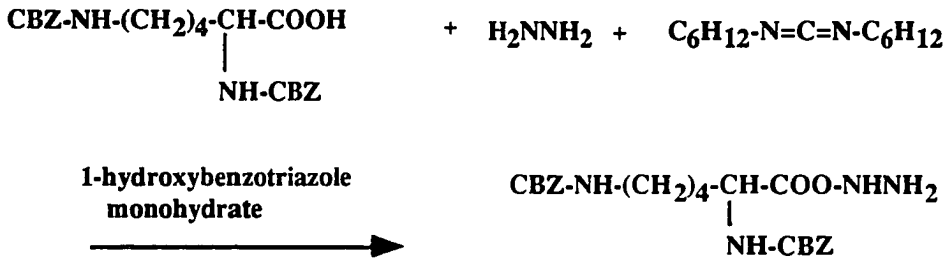
Develop the product from Step 5 on pre-coated TLC plates (silica Gel 60, silanized) with a solution of methanol:water (5:1). $R_{ala} = 0.52$, $Rala_5$ and $Rala_6 = 0.61$.

7. Purification by HPLC

HPLC conditions are similar to Step 7 of ^{3}H-glu$_2$ synthesis. Collect the ^{3}H-ala$_6$-containing fraction of the eluate between 5.5 and 8 minutes.

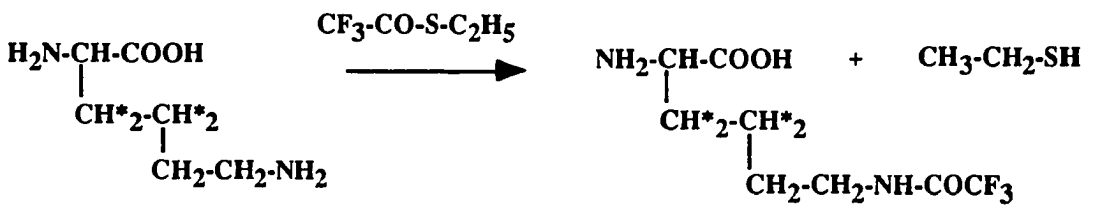
V. Synthesis of ^{3}H-lys$_2$ from di-N-CBZ-lys and ^{3}H-lys

1. Preparation of hydrazide



Procedure is similar to Step 1 of ^{3}H-glu$_2$ synthesis.

2. Preparation of N-CF$_3$CO-^{3}H-lys

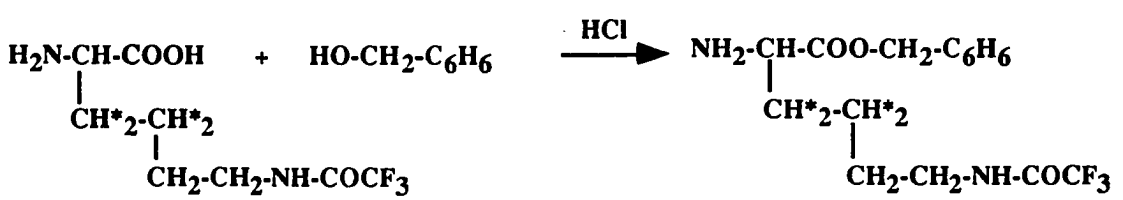


(1) Dry 120 μL of ^{3}H-lys [3,4-^{3}H] (0.12 mCi, 0.002 mmol) in 0.01 N HCl solution *in vacuo*.

(2) Add 1 N NaOH (100 μL) and ethyl thioltrifluoroacetate (20 μL, 0.158 mmol), then stir at room temperature for 6 hours.

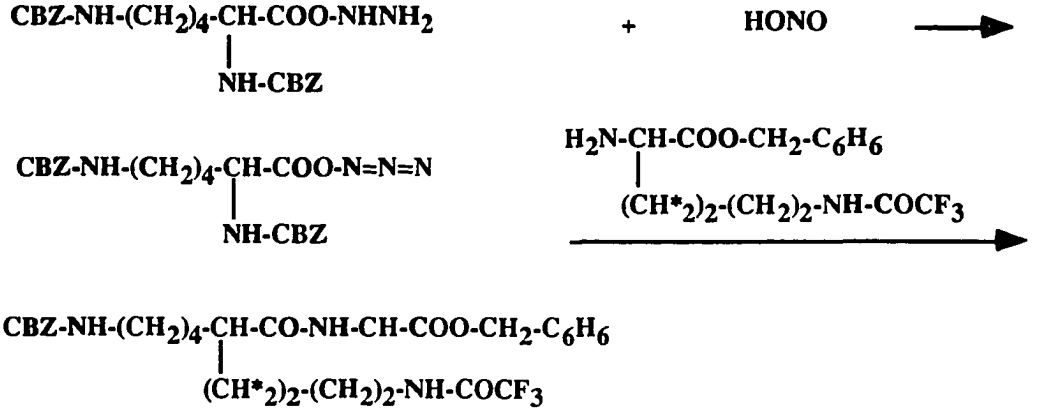
(3) Remove the solvent *in vacuo*.

3. Preparation of N-CF$_3$CO-^{3}H-lys benzyl ester



Add 60 μL benzyl alcohol to dissolve the residue from Step 2. The rest of the procedure is similar to Step 2 of ^{3}H-glu$_2$ synthesis.

4. Conversion of the hydrazide to the azide and coupling of the benzyl ester with the azide



Procedure is similar to Step 3 of ^{3}H-glu$_2$ synthesis.

5. Deprotection of N-CF_3CO-group

(1) Dissolve the residue from Step 4 in 100 μL 80% aqueous piperidine.

(2) Stir for 2 hours at room temperature.

(3) Dry *in vacuo* .

6. Deprotection of the benzyl ester (CBZ) group by hydrogenation

Procedure is similar to Step 5 of ^{3}H-glu$_2$ synthesis.

7. Purification by TLC

Develop the product from Step 6 on pre-coated TLC plates (silica Gel 60, silanized) with a solution of methanol:formic acid:water (77:2:28). $R_{lys} = 0.60$, $R_{lys2} = 0.46$.

8. Purification by HPLC

HPLC conditions are similar to Step 7 of ^{3}H-glu$_2$ synthesis. Collect the ^{3}H-lys$_2$-containing fraction of the eluate between 8 and 12 minutes.

APPENDIX III: Tables of Experimental Data

Table A.1. Relative Molar Response Factors of Amino Acids and Peptides

aaa	1.00
ala	1.311±0.051 (n = 4)
ala_2	0.134±0.013 (n = 4)
ala_3	0.082±0.020 (n = 6)
ala_5	0.054±0.008 (n = 14)
glu	1.172±0.038 (n = 4)
glu_2	0.051±0.008 (n = 4)
lys	0.573±0.015 (n = 4)
lys_2	0.173±0.034 (n = 13)

Table A.2. Several Measurements of Adsorption and Hydrolysis in Skan Bay Sediment

peptide	concentration in pore water (μM)	^{3}H-activity added (x10^3 dpm/mL)	sediment* (n)	% of added ^{3}H-peptide adsorbed	hydrolyzed	remaining
lys_2	0.01	8.5	fresh I(3)	16.1±3.1	25.5±2.6	71.9±11.2
		8.0	stored I(3)	15.5±3.5	26.7±1.3	57.8±2.3
		7.2	stored II(4)	9.9±2.8	26.1±0.9	64.0±1.9
	0.03	8.3	fresh I(3)	15.2±2.2	28.9±8.7	63.4±1.5
		0.8	fresh II(3)	13.2±0.8	36.9±0.4	49.6±0.5
	3.33	0.8	fresh II(3)	16.1±3.1	25.5±2.6	64.0±4.9
		8.3	stored II(6)	20.4±7.2	12.4±1.2	63.0±3.8
ala_2	0.01	5.7	fresh I(3)	1.8±1.1	19.4±0.5	29.6±4.2
		6.0	stored I(3)	1.7±1.1	19.4±0.5	78.9±0.6
	0.03	5.7	fresh I(3)	10.6±0.4	53.3±4.3	33.6±1.9
		0.5	fresh II(3)	13.2±4.1	49.0±3.3	37.8±0.9
	3.33	0.5	fresh II(3)	25.1±3.4	31.9±2.2	50.1±4.3
		5.8	stored I(3)	28.0±3.3	30.0±2.2	43.0±1.2
		6.7	stored II(6)	22.8±8.9	5.6±3.8	71.6±5.1
ala_3	0.01	4.2	fresh I(3)	15.4±3.0	6.2±1.2	77.8±3.8
		10.3	fresh I(3)	15.9±2.1	5.2±0.4	79.0±1.6
		10.8	stored II(4)	26.5±3.1	3.3±0.7	70.2±2.4
	0.03	4.1	fresh I(3)	0.0	28.4±1.4	93.3±3.2
		0.4	fresh II(3)	0.0	0.0	118±1.0
	3.33	0.4	fresh II(3)	0.0	26.0±2.1	99.0±5.0
		10.9	fresh I(3)	32.0±0.9	1.7±0.2	69.2±0.7
		10.3	stored II(6)	27.3±5.4	4.0±1.6	68.6±4.1

* Fresh I and II stand for fresh sediments from the different cores. Stored I and II stand for sediments stored about two months which originated from the same core, but were used in experiments conducted at different times.

Table A.3. Decomposition of ^{3}H-Peptides in Skan Bay Sediment

sediment	peptide	concentration of solution (μM)	% of ^{3}H-peptide added: hydrolyzed ^{3}H-FAA	respired ^3H_2O	adsorbed
stored[1]	lys_2	0.03	51.4±1.6	0.0	15.7±2.4
		3.33	46.6±2.0	0.0	22.7±3.2
	glu_2	0.03	79.9±10.1	0.0	0.0
		3.33	64.8±8.2	5.3±0.2	9.8±3.5
	ala_2	0.03	65.6±3.9	0.0	1.7±2.4
		3.33	56.6±9.3	0.7±0.8	16.2±1.2
	ala_3	0.03	69.4±17.4	0.0	2.2±2.6
		3.33	68.4±1.5	0.0	13.6±1.9
	ala_6	0.03	59.7±4.2	0.0	12.1±4.7
		3.33	74.3±4.1	0.0	6.2±4.0
fresh[2]	lys_2	0.01	25.5±2.6	1.1±0.2	16.1±3.1
		0.03	28.9±8.7	0.4±0.0	15.2±2.2
		3.33	12.3±2.0	3.3±0.3	20.4±7.2
	glu_2	0.01	75.6±13.2	1.1±0.2	11.0±2.7
		0.03	60.8±7.8	1.1±0.3	14.5±10.2
		3.33	73.1±5.1	0.0	1.7±1.4
	ala_2	0.01	19.4±0.5	0.0	1.8±1.1
		0.03	53.3±4.3	0.0	10.6±0.4
		3.33	31.9±2.2	2.5±5.1	25.1±3.4
	ala_3	0.01	6.2±1.2	0.0	15.4±3.0
		0.03	28.4±1.4	0.0	0.0
		3.33	1.7±0.2	0.0	32.0±0.9
	ala_6	0.01	6.0±1.2	0.0	6.4±4.4
		0.03	32.5±3.4	0.7±0.9	0.0
		3.33	4.8±0.8	0.7±1.7	9.3±2.5

[1] Sediment was collected in July, 1990 and studied in March 1992.
[2] Measurements made with fresh sediment on board ship data in October, 1992.
[3] Incubation time was 18 min.

Table A.4. Decomposition and Adsorption of ^{3}H-Peptides in Resurrection Bay Sediment

sediment	peptide	concentration of solution (μM)	% of ^{3}H-peptide added: hydrolyzed ^{3}H-FAA	respired ^3H_2O	adsorbed
stored[1]	lys_2	0.03	13.6±5.2	0.0	25.9±3.7
		3.33	20.2±1.2	0.0	18.1±4.2
	glu_2	0.03	83.4±1.7	0.6±1.1	5.6±3.8
		3.33	83.8±2.8	2.3±2.8	3.8±3.9
	ala_2	0.03	70.9±3.7	0.0	3.6±4.3
		3.33	70.9±2.5	10.7±0.3	4.1±3.1
	ala_3	0.03	58.4±0.2	1.9±0.0	25.3±1.0
		3.33	65.7±2.3	0.0	12.7±3.1
	ala_6	0.03	23.3±1.9	1.5±0.2	40.9±4.2
		3.33	23.4±2.5	0.0	31.2±4.2
fresh[2]	lys_2	0.03	14.1±3.5	0.4±0.1	17.7±4.9
		3.33	20.5±9.9	0.1±0.1	25.2±0.8
	glu_2	0.03	18.0±2.9	0.7±0.1	25.6±0.8
		3.33	14.7±3.9	0.5±0.0	25.3±2.4
	ala_2	0.03	21.1±3.1	3.2±0.1	31.9±13.0
		3.33	58.4±3.6	22.2±2.4	29.7±2.4
	ala_3	0.03	0.0	0.0	32.0±1.6
		3.33	47.3±2.7	3.3±0.4	16.1±3.2
	ala_6	0.03	3.1±2.8	0.7±0.1	25.0±4.8
		3.33	23.3±10.9	0.0	18.5±2.3

[1] Sediments were collected in September, 1989 and studied in March 1992.
[2] Measurements made with fresh sediment on board ship data in May, 1993.
[3] Incubation time was 18 min.

Table A.5. Activity Recovery in Skan Bay Sediment

Autoclaved Sediment

peptide		% of added activity*				
		pore water	1st acid extract	sea water extract	2nd acid extract	Σ recovery
μM						
lys_2	A	71.5	2.8	12.2	8.1	94.6
3.33	B	72.1	4.6	7.5	10.9	95.1
	C	70.3	3.5	9.0	47.1	129.9
glu_2	A	98.5	0.0	0.0		98.5
3.33	B	90.4	0.0	6.7		97.1
	C	94.0	2.6	0.0		96.6
ala_2	A	77.1	12.4	4.5		94.0
3.33	B	86.9	10.1	0.0		97.0
	C	81.4	12.3	2.9		96.6
ala_3	A	80.7	10.8	9.5		101.8
3.33	B	74.4	12.1	16.2		102.7
	C	69.6	13.0	12.9		95.5
ala_6	A	79.2	2.3	11.9		93.4
3.33	B	83.3	0.0	10.5		93.8
	C	89.2	0.0	9.3		98.5

Table A.5 (continued)

Untreated Sediment

peptide		% of added activity				
μM		pore water	sea water extract	acid extract	hot acid hydrolysis	Σ recovery
lys_2	A	85.0	2.5	12.9		100.4
0.03	B	79.1	0.0	14.3		93.4
	C	78.4	0.0	18.0		96.4
ala_2	A	63.1	27.4	0.1	8.1	98.7
0.03	B	69.5	20.3	0.9		90.7
	C	73.0	18.6	0.4		92.0
ala_3	A	57.2	35.7	0.6	11.9	105.4
0.03	B	54.7	37.0	0.0		91.7
	C	52.9	36.2	1.7		90.8

*% pore water = activity that remained in solution
% seawater extract = activity recovered with 5 mL seawater rinse (3 times)
% acid extract = activity extracted with 5 mL 1 N HCl
% 1st acid extract = activity extracted with 3 mL 1 N HCl
% 2nd acid extract = activity extracted with 5 mL 1 N HCl (2 times)
% hydrolysis = activity recovered with 6N HCl hydrolysis at 110°C for 12 h

Table A.6. Comparison of Means of Adsorption Data in Untreated and Autoclaved Sediments using F Statistic Test[1] with $\alpha = 0.05$

peptide	concentration	sediment				F test with α=0.05						
	(μM)	location[2]	treatment	n	$\bar{y}$	υ_1	υ_2	MST	MSE	F		F_α
ala_2	0.03	SB	untreated	9	8.82							
			autoclaved	6	2.38							
						1	13	149.30	34.85	4.28	<	4.67
	3.33		untreated	6	2.45							
			autoclaved	3	2.93							
						1	7	0.47	13.34	0.04	<	5.59
	0.03	RB	untreated	15	22.40							
			autoclaved	2	21.50							
						1	15	1.43	252.91	0.01	<	4.54

Table A.6. (continued)

peptide	concentration (μM)	sediment location**	treatment	n	$\bar{y}$	F test with α=0.05 υ_1	υ_2	MST	MSE	F		F_α
ala_3	0.03	SB	untreated	10	10.58							
			autoclaved	3	0.0							
						1	11	258.00	56.87	4.54	<	4.84
	3.33		untreated	6	4.52							
			autoclaved	3	0.0							
						1	7	40.86	18.73	2.18	<	5.59
ala_6	0.03	SB	untreated	9	1.44							
			autoclaved	6	0.0							
						1	13	7.51	8.90	0.84	<	4.67
	3.33		untreated	6	4.70							
			autoclaved	3	0.0							
						1	7	44.09	20.98	2.10	<	5.59
	0.03	RB	untreated	15	19.50							
			autoclaved	2	17.70							
						1	15	5.72	66.78	0.09	<	4.54

Table A.6. (continued)

peptide	concentration	sediment				F test with α=0.05					
	(μM)	location**	treatment	n	$\bar{y}$	υ_1	υ_2	MST	MSE	F	F_α
glu_2	0.03	SB	untreated	12	3.91						
			autoclaved	6	0.42						
						1	16	48.72	20.45	2.38 <	4.49
	3.33		untreated	3	1.65						
			autoclaved	3	2.02						
						1	4	0.21	7.15	0.03 <	7.71
	0.03	RB	untreated	3	15.30						
			autoclaved	2	9.05						
						1	3	46.88	13.91	3.37 <	10.13

Table A.6. (continued)

peptide	concentration	sediment				F test with α=0.05						
	(μM)	location**	treatment	n	$\bar{y}$	υ_1	υ_2	MST	MSE	F		F_α
lys_2	0.03	SB	untreated	13	13.14							
			autoclaved	6	14.43							
						1	17	6.83	19.89	0.34	<	4.45
	3.33		untreated	9	22.36							
			autoclaved	3	14.23							
						1	10	148.72	31.18	4.77	<	4.96
	0.03	RB	untreated	15	12.63							
			autoclaved	2	18.95							
						1	15	11.15	49.00	0.23	<	4.54

[1] In F test (Mendenhall 1987), n = number of measurements, n_1 = untreated and n_2 = autoclaved;
$\bar{y}$ = mean of each measurement (% of ^{3}H-peptide adsorption); $\upsilon_1 = 1$ and $\upsilon_2 = (n_1 + n_2 - 2)$;

$$MST = \frac{n_1 n_2}{n_1 + n_2}(\bar{y}_1 - \bar{y}_2)^2 \frac{1}{\upsilon_1}\ ;\ MSE = \langle \sum_{j=1}^{n_1} (\bar{y}_{1j} - \bar{y}_1)^2 + \sum_{j=1}^{n} (\bar{y}_{2j} - \bar{y}_2)^2 \rangle \frac{1}{\upsilon_2};\ F = \frac{MST}{MSE};$$

if $F < F_\alpha$ (the critical value of the F statistic for $\alpha = 0.05$ at υ_1 and υ_2), there is not sufficient evidence to indicate a difference between the two means in untreated and autoclaved sediments.
[2] SB = Skan Bay, RB = Resurrection Bay.

Table A.7. Amino Acid Enantiomers in Acid Hydrolyzed ^{3}H-Peptides

^{3}H-amino acid hydrolyzed from ^{3}H-peptide[1]	^{3}H-amino acid enantiomer[2]	
	% of D-amino acid	% of L-amino acid
glutamic acid from glu_2	7.4	92.6
alanine from ala_2	0	100
alanine from ala_3	0	100
alanine from ala_6	0	100

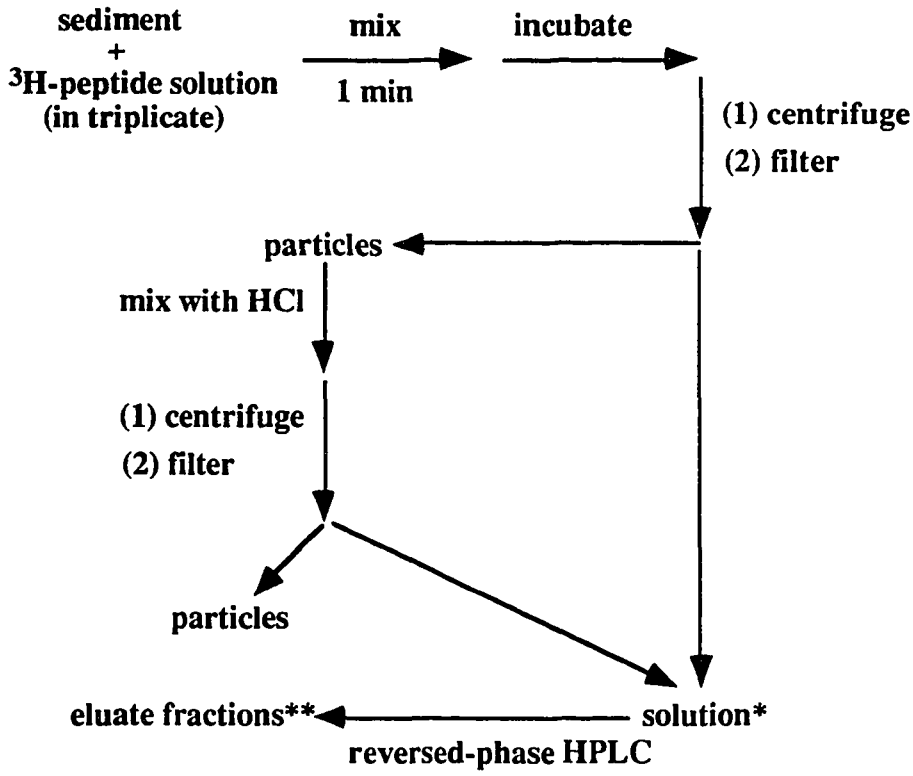
[1] ^{3}H-peptides were hydrolyzed in 6N HCl, at 110°C for 20 h (Robertson *et al.* 1987). 92% to 100% of the ^{3}H-activity was recovered as ^{3}H-amino acids after acid hydrolysis.

[2] The resolution of enantiomers of hydrolyzed amino acids were conducted by reversed-phase HPLC using Lichrosorb RP-18 column with the mobile phase of 13% acetonitrile in an aqueous solution containing 1 mM *N*-(*p*-toluene-sulfonyl)-L-phenylalanine and 0.5 mM $CuSO_4 \cdot 5H_2O$, pH 5-6 (Nimura *et al.* 1983). Resolution values (Rs) for glutamic acid and alanine enantiomers are 0.67 and 0.36, respectively.

Table A.8. Constant Terms in the Equations of the Tracer Model

sediment	^{3}H-peptide	constant term		
		B_2	B_3	B_4
Skan Bay	lys_2	91	-486	-11171
	glu_2	6	249	915
	ala_2	9	2032	2
	ala_3	5	-266	5
	ala_6	6	-36	3
Resurrection Bay	lys_2	28	-288	-2361
	glu_2	8	-72	194
	ala_2	4	-154	-101
	ala_6	23	-13	-1749

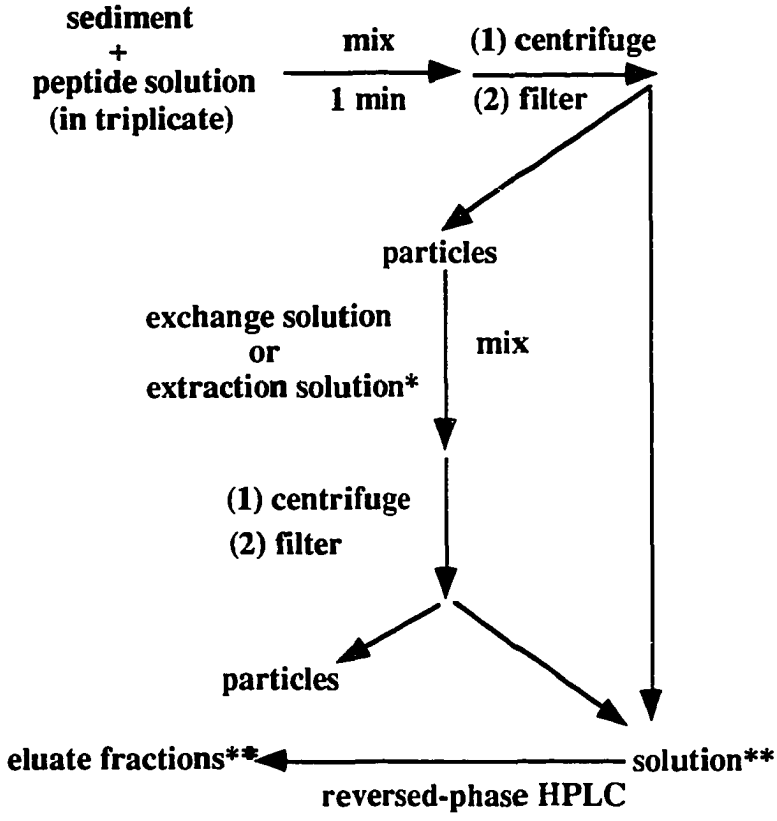
APPENDIX IV: Figures Illustrating Experimental Methods and Data



*The ^3H_2O in solution was recovered by distillation and its activity was assayed separately.

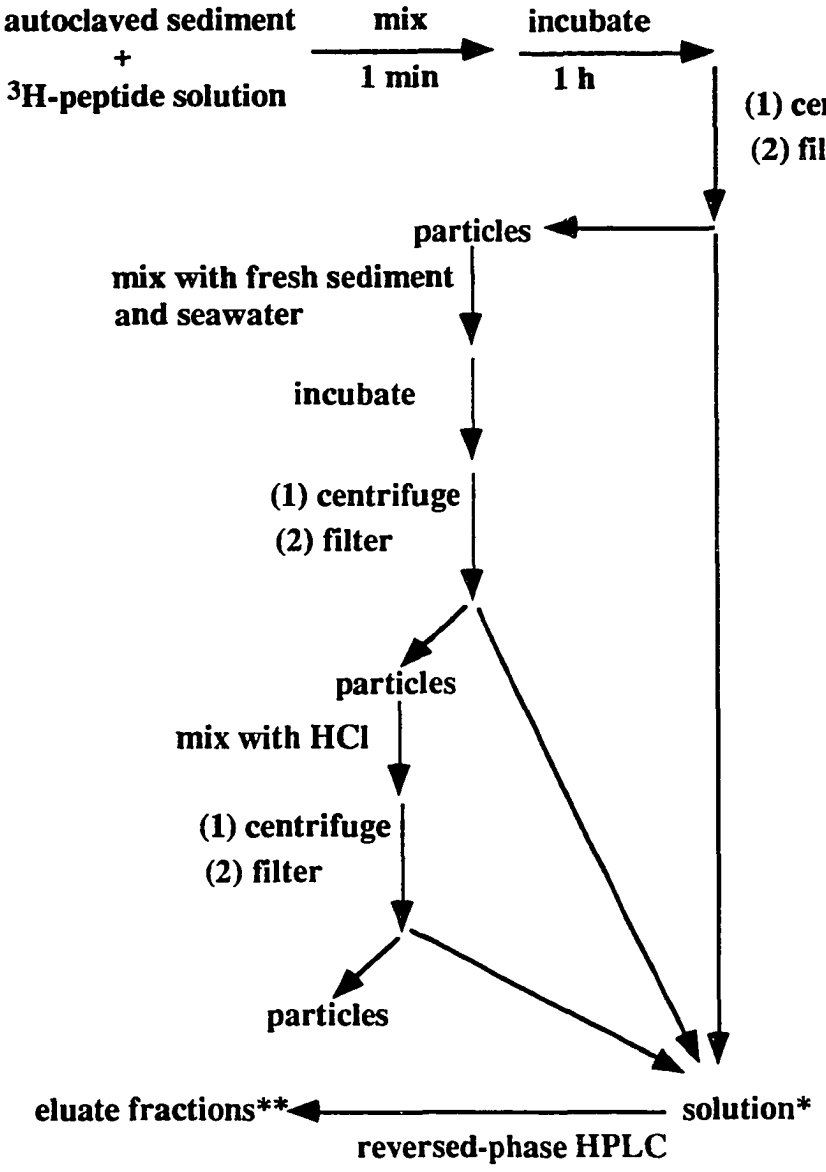
** The activity of ^{3}H-peptide and ^{3}H-free amino acid-containing fractions was measured.

Figure A.1a. Schematic of Decomposition Experiment.



* Exchange solutions are seawater, sodium (acetate) and (cesium) chloride. Extraction solutions are HCl and NaOH.
* *See Figure A.1a.

Figure A.1b. Schematic of Adsorption, Exchange and Extraction Experiments.



*See Figure A.1a.
** See Figure A.1a.

Figure A.1c. Schematic of Decomposition Experiment for Adsorbed Peptides.

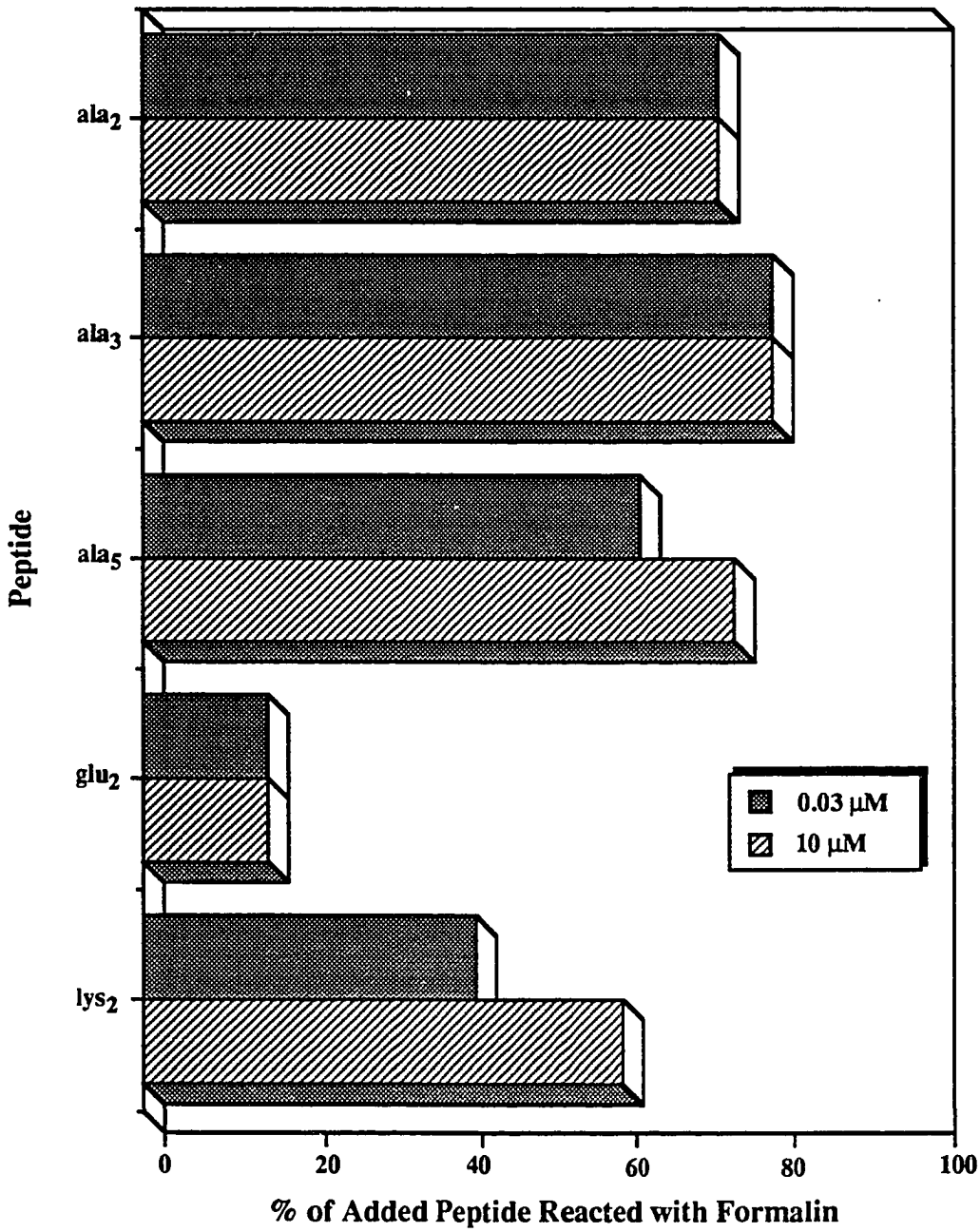


Figure A.2. Reaction Between Peptide and Formalin in Pore Water from Skan Bay Sediment.

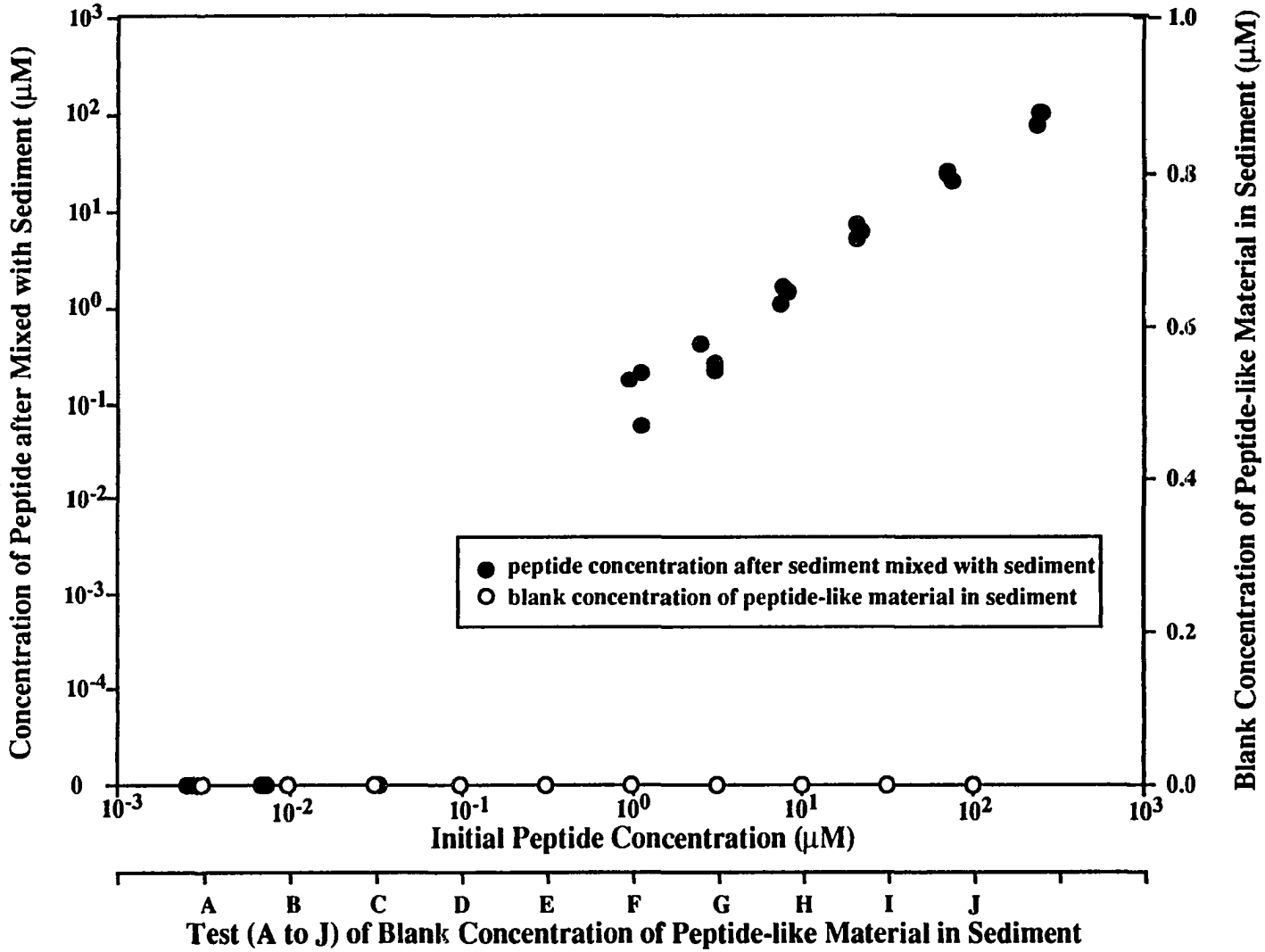


Figure A.3a. Concentrations of Ala_2 in Pore Water from Skan Bay Sediment (Formalin Treated).

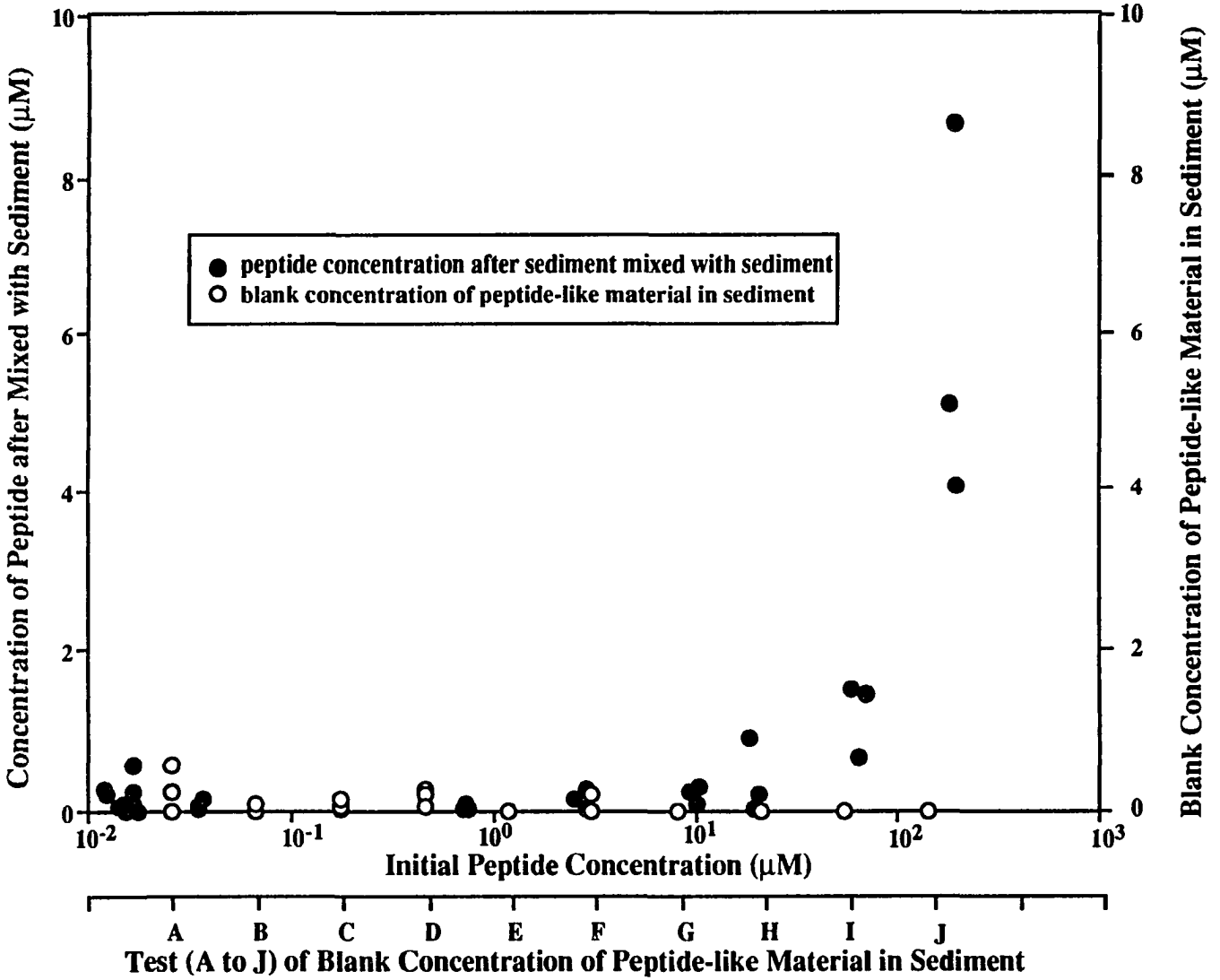


Figure A.3b. Concentrations of Ala_3 in Pore Water from Skan Bay Sediment (Formalin Treated).

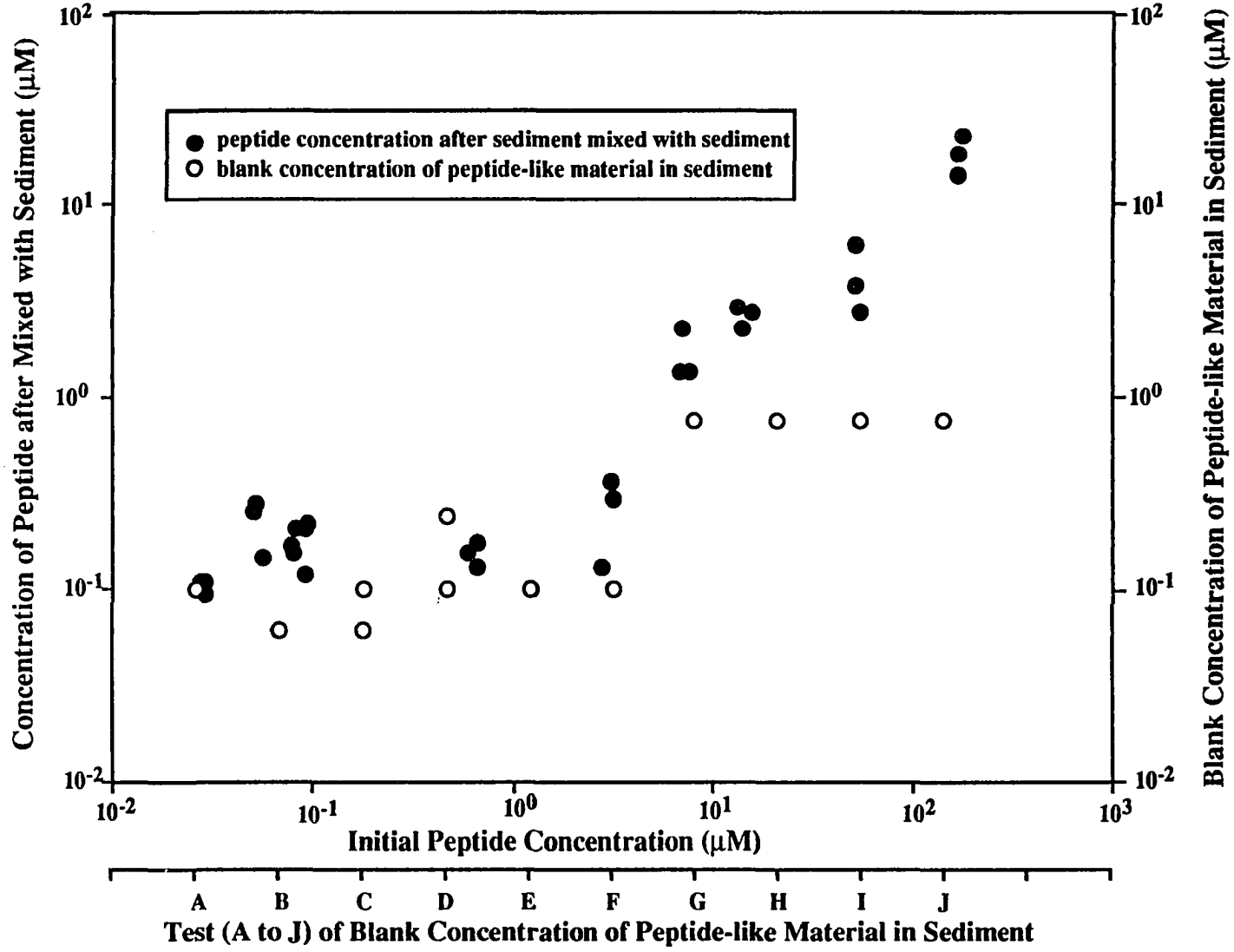


Figure A.3c. Concentrations of Ala_5 in Pore Water from Skan Bay Sediment (Formalin Treated).

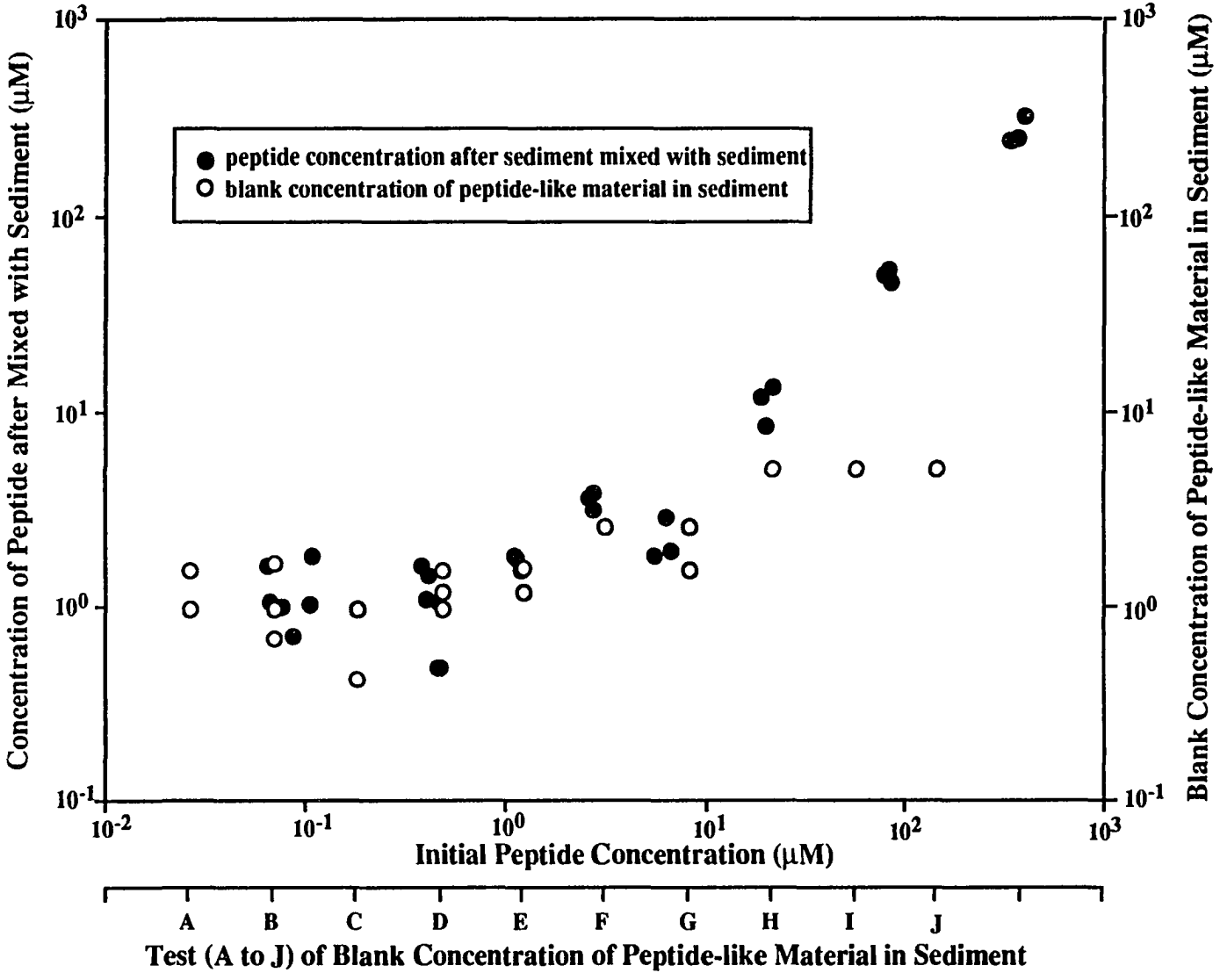


Figure A.3d. Concentrations of Glu_2 in Pore Water from Skan Bay Sediment (Formalin Treated).

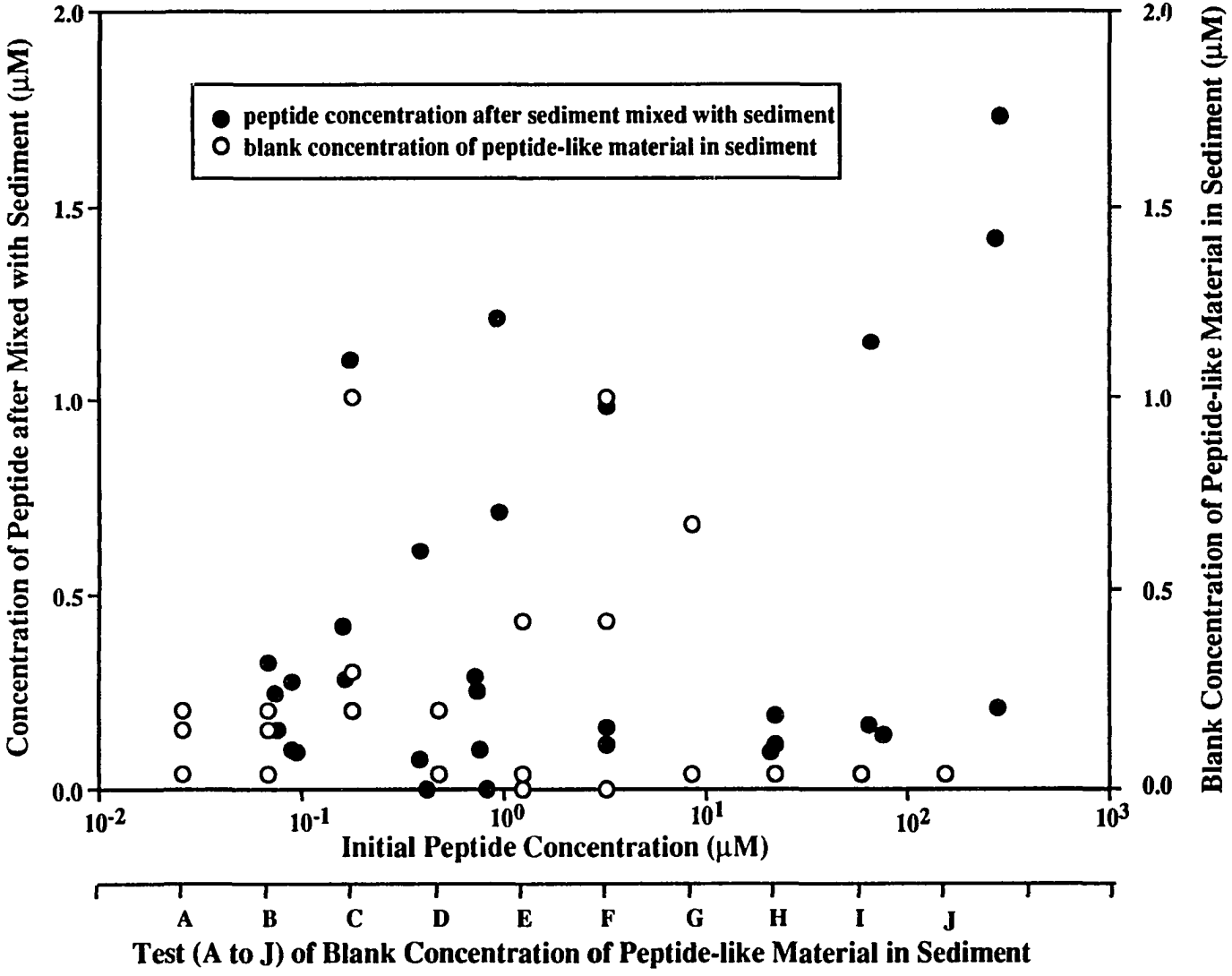


Figure A.3e. Concentrations of Lys_2 in Pore Water from Skan Bay Sediment (Formalin Treated).

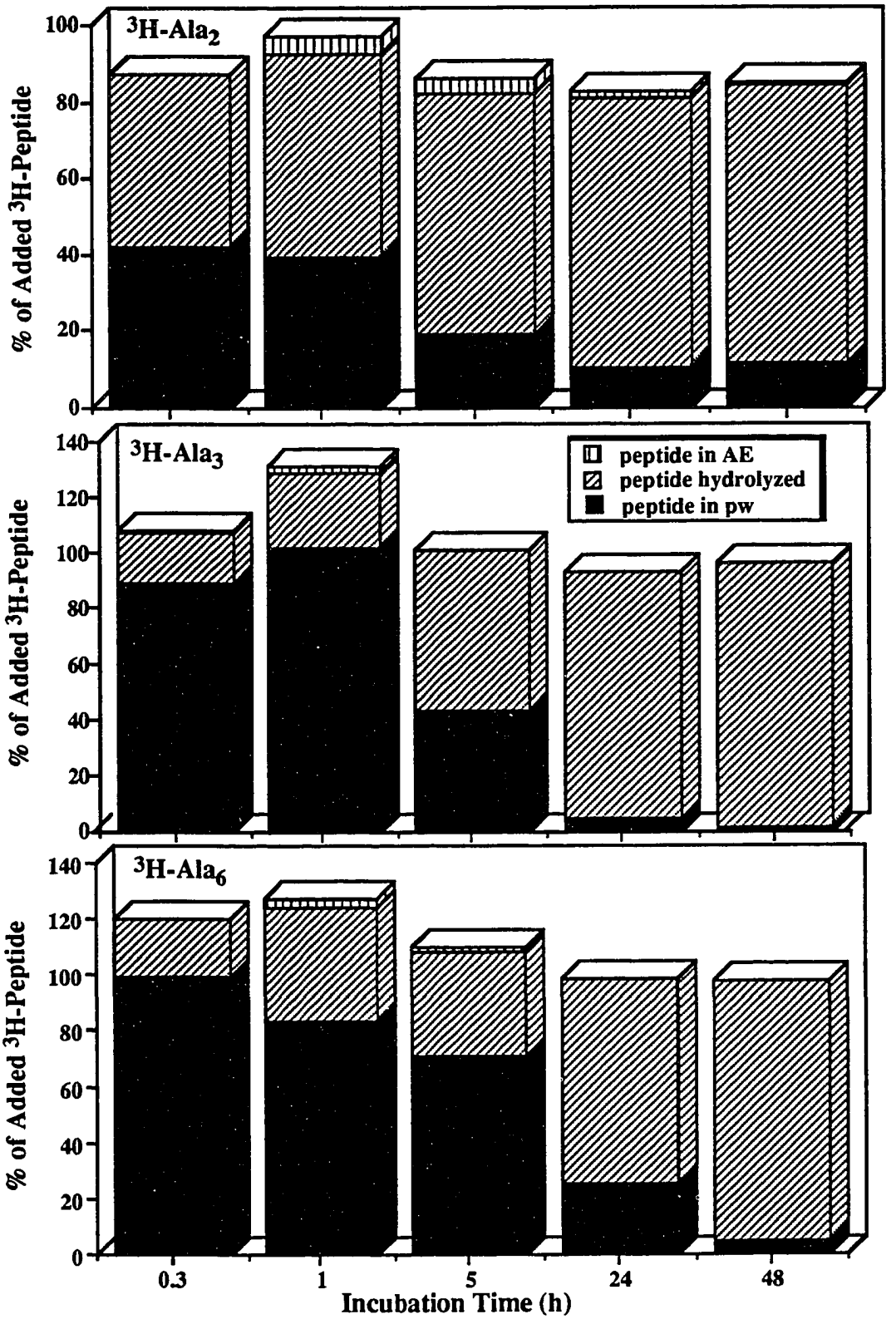


Figure A.4. Recovery of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Skan Bay Sediment.

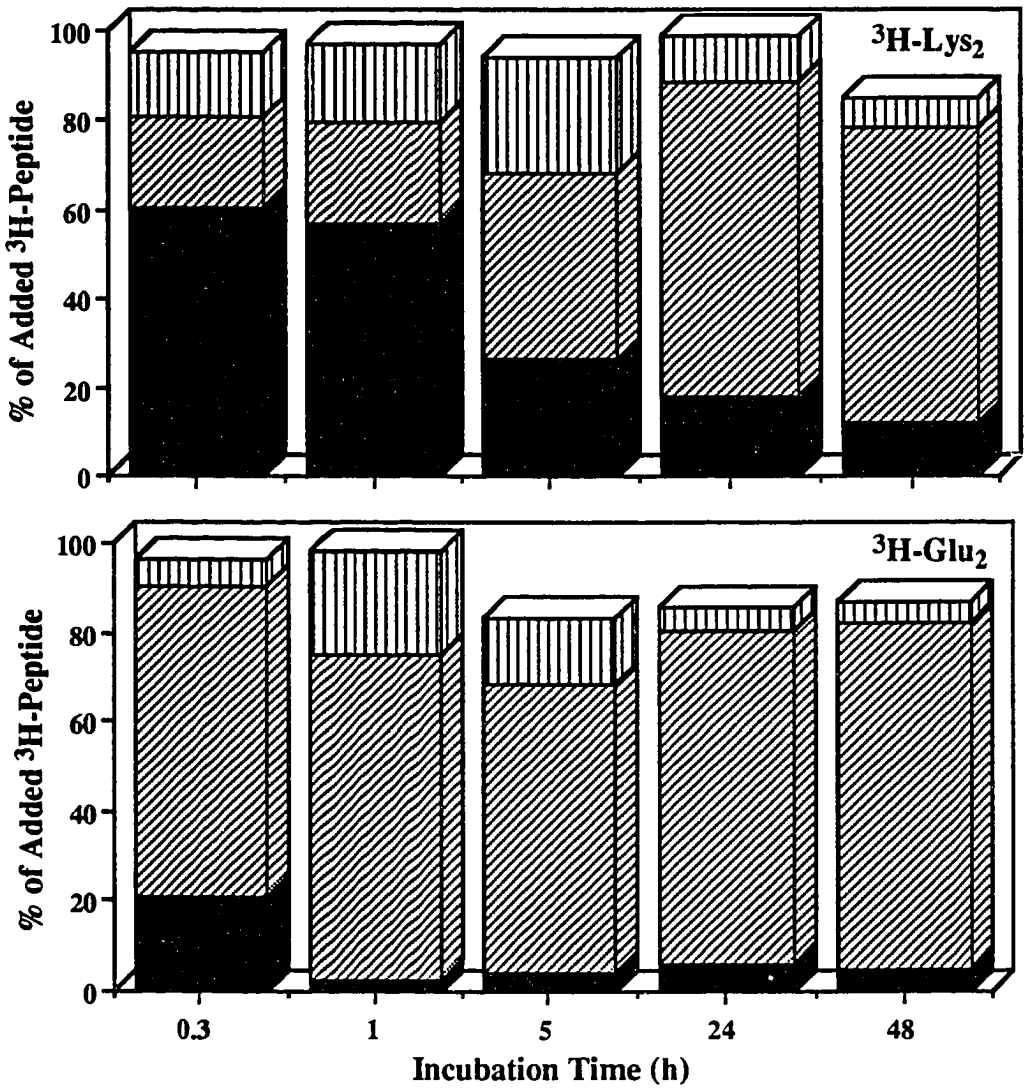


Figure A.4. (continued)

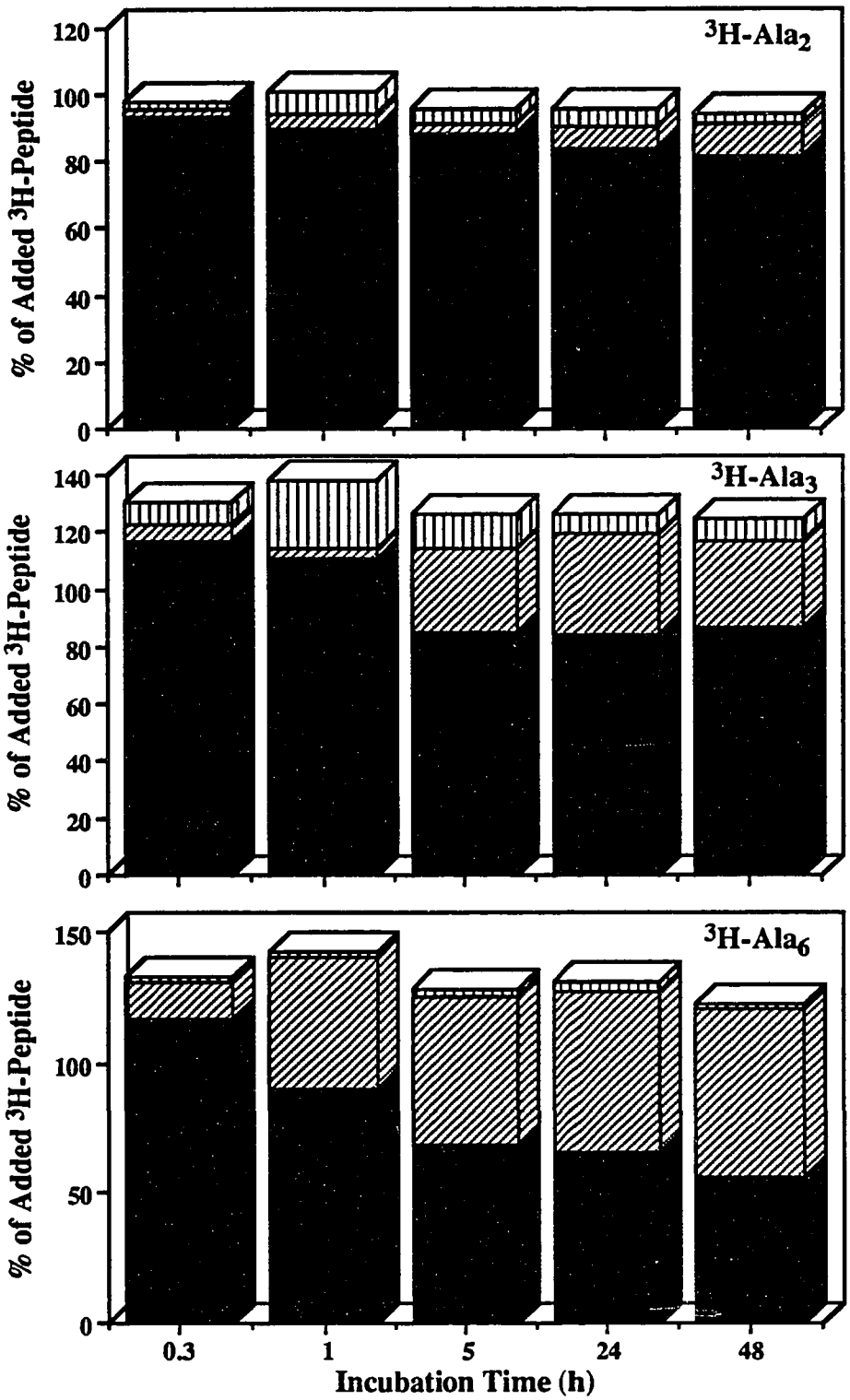


Figure A.5. Recovery of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Skan Bay Sediment (Autoclaved).
▯ peptide in AE ▨ peptide hydrolyzed ■ peptide in pw

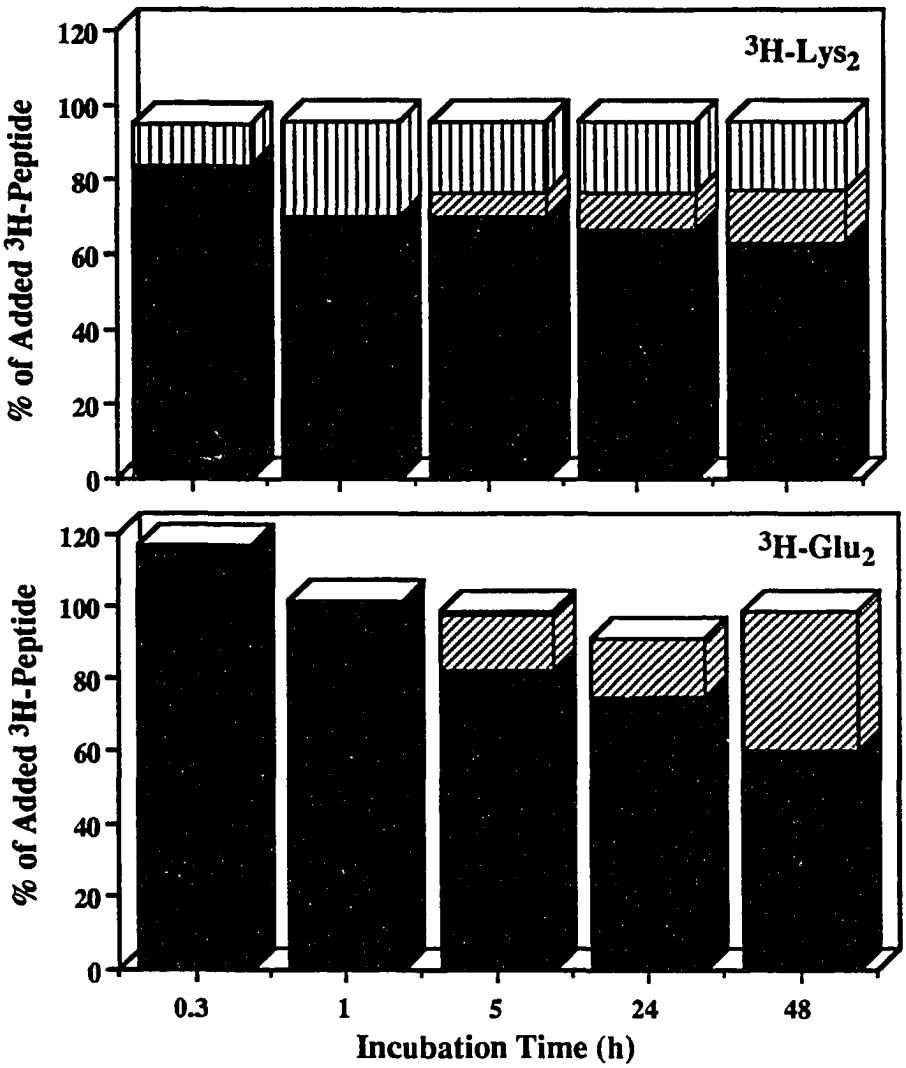


Figure A.5. (continued)

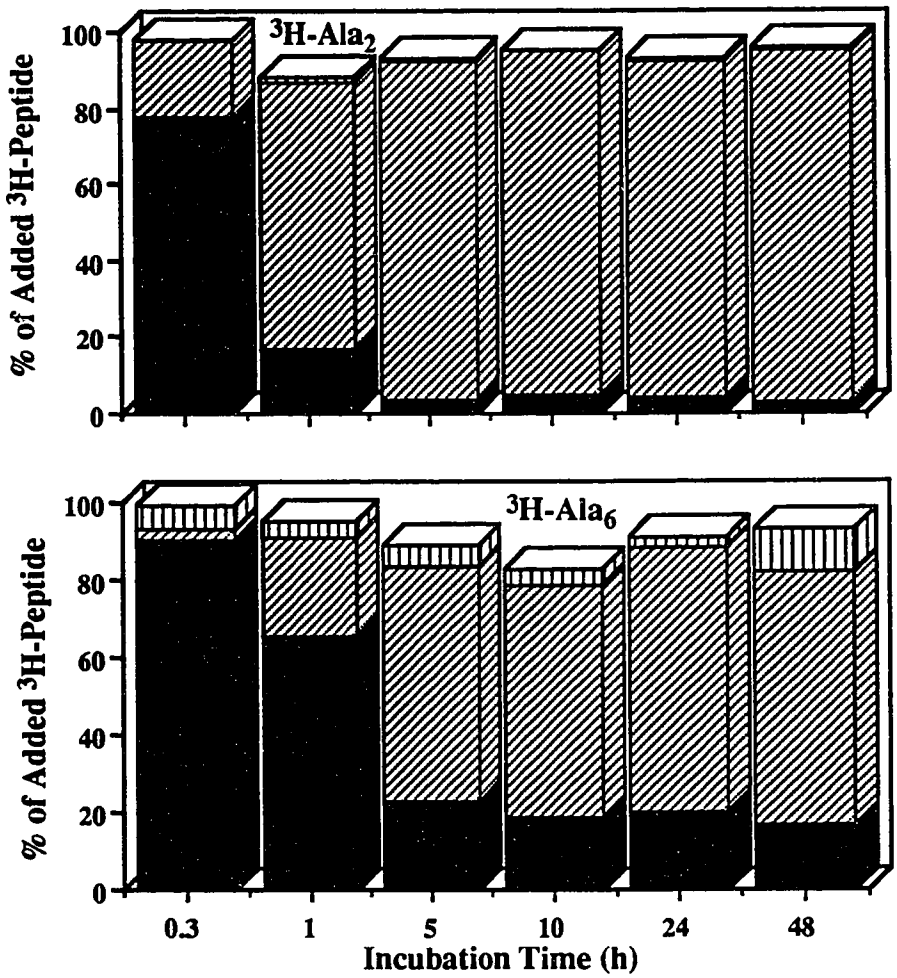


Figure A.6. Recovery of ^{3}H-Peptides (at 0.03 μM Initial Concentration) in Resurrection Bay Sediment.
▯ peptide in AE ▨ peptide hydrolyzed
■ peptide in pw

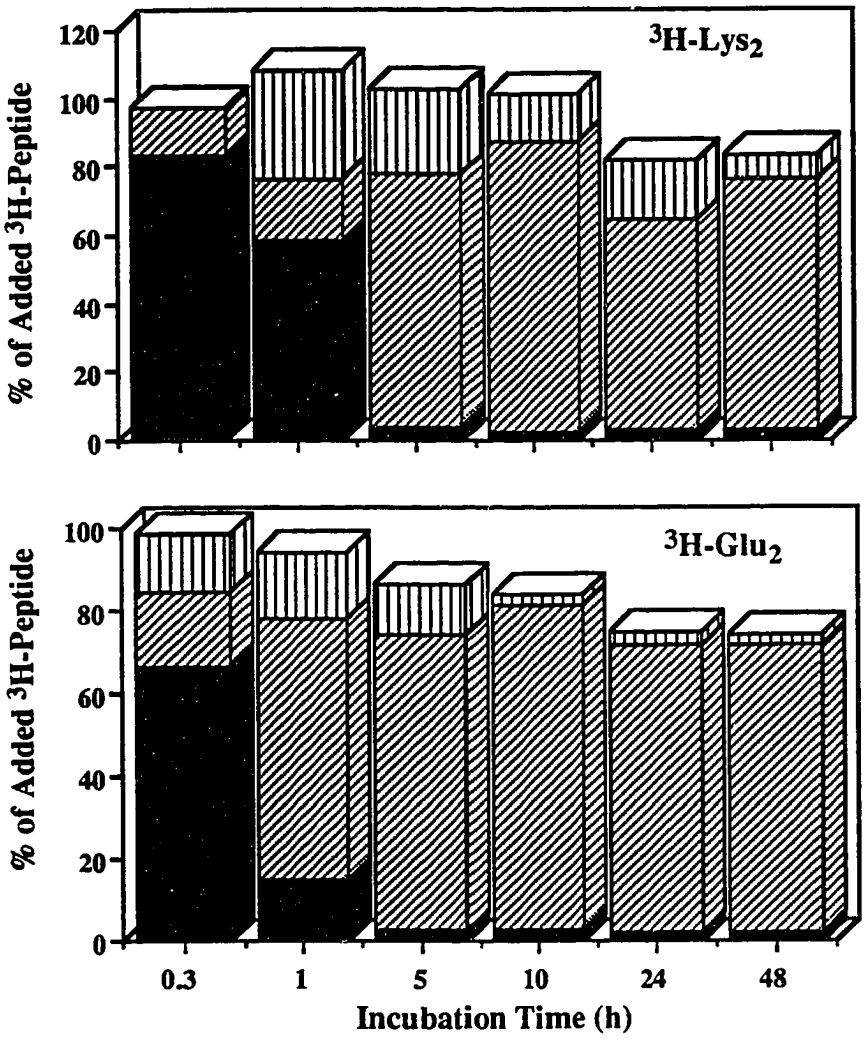


Figure A.6. (continued)

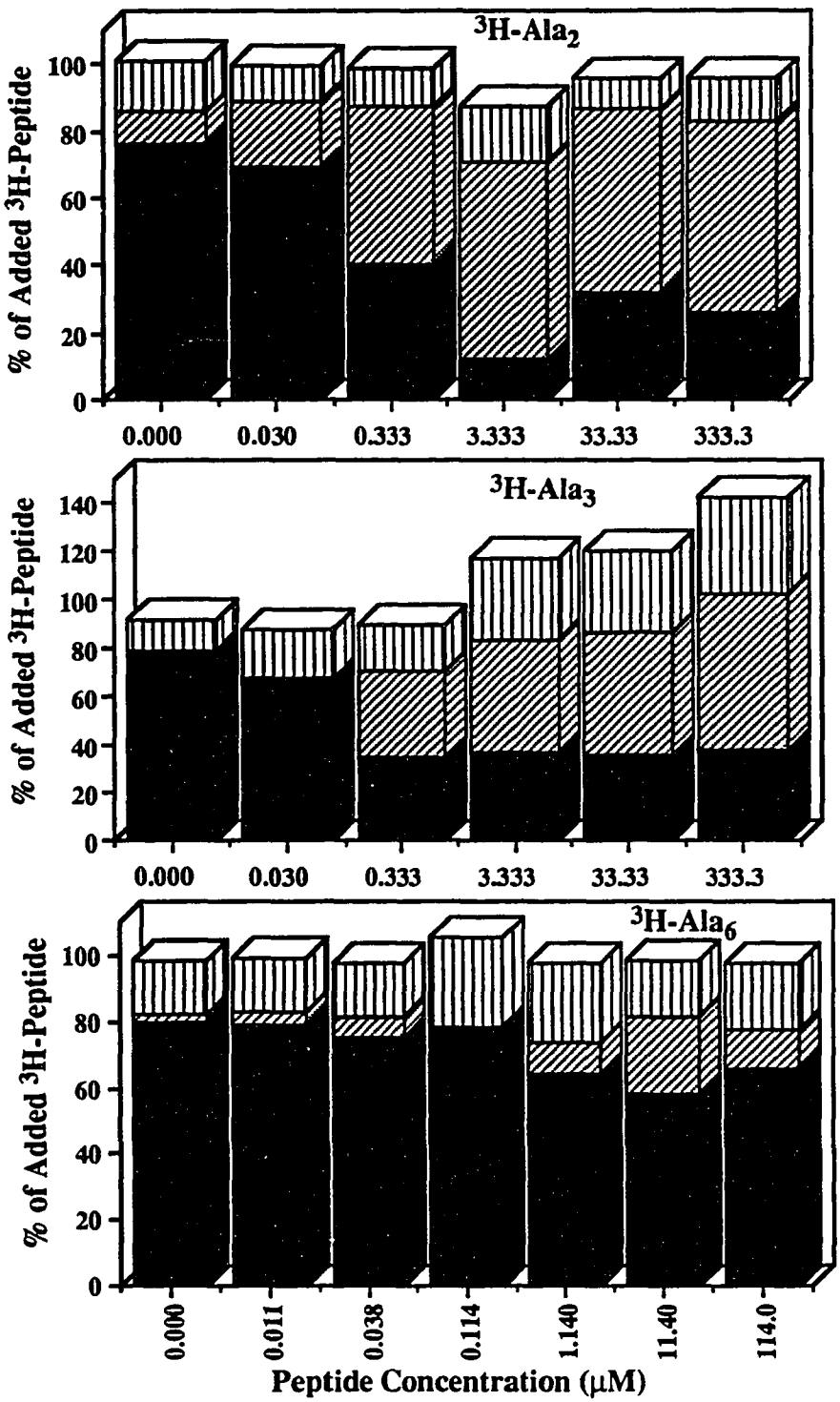


Figure A.7. Recovery of ^{3}H-Peptides in Resurrection Bay Sediment.

▯ peptide in AE ▨ peptide hydrolyzed

■ peptide in pw

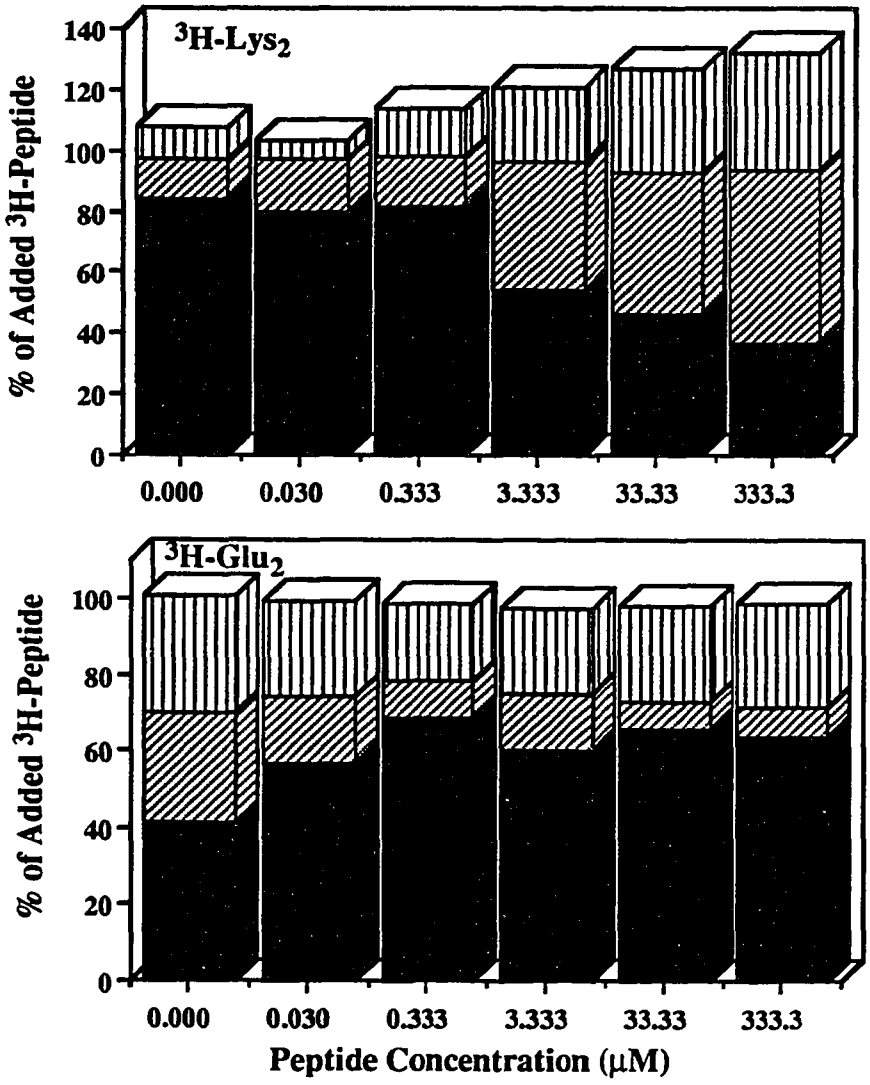


Figure A.7. (continued)